

FIG.1

# FIG.2A

LOCUS HSU22027 7215 bp DNA 22-OCT-1995  
 DEFINITION Human cytochrome P450 (CYP2A6V2) gene, complete cds.  
 ACCESSION U22027  
 NID g1008461  
 KEYWORDS  
 SOURCE human.  
 ORGANISM Homo sapiens  
 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;  
 Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.  
 REFERENCE 1 (bases 1 to 7215)  
 AUTHORS Fernandez-Salguero, P., Hoffman, S.M., Cholerton, S., Mohrenweiser, H.,  
 Raunio, H., Rautio, A., Pelkonen, O., Huang, J.D., Evans, W.E.,  
 Idle, J.R. et, al.  
 TITLE A genetic polymorphism in coumarin 7-hydroxylation: sequence of the  
 human CYP2A genes and identification of variant CYP2A6 alleles  
 Am. J. Hum. Genet. 57 (3), 651-660 (1995)  
 JOURNAL 95397851  
 MEDLINE  
 REFERENCE 2 (bases 1 to 7215)  
 AUTHORS Fernandez-Salguero, P.  
 TITLE Direct Submission  
 JOURNAL Submitted (01-MAR-1995) Pedro Fernandez-Salguero, National  
 Institutes of Health, 9000 Rockville Pike, Bethesda, MD 20894, USA  
 FEATURES Location/Qualifiers  
 source 1..7215  
 /organism="Homo sapiens"

FIG.2A CONT.

5'UTR  
CDS

782..790  
join (791..970, 1237..1399, 2115..2264, 2499..2659,  
3207..3383, 4257..4398, 4873..5060, 5577..5718, 6308..6489)  
/gene=CYP2A6V2:  
/codon\_start=1  
/product=cytochrome P450"  
/db\_xref-PID:g1008462"  
/translation=MLASGMLLVALLACTVMLMSVWQKSKGKLP PGP TPLPFIG  
NYLQNT EQMYNSLMKISERYGPVFTIHLGPRRVVLCGHDAVREALVDOAE EFSGRG  
EQATFDWVFKGYGVVFSNGERAKQLLRFAIATLRDFGVGKRGI EERIQEESGFLIEAI  
RSTHGANIDPTFFLSRTVSNVISSIVFGDRFDYKDKEFLSLRMLGLIFQFTSTSTGQ  
LYEMFSSVMKHLPGPQQQAFQLLQGLEDFIAKKVEHNQRTLDPN SPRDFIDSLIRMQ  
EEKNPNT E FYLKNLMMSTLNLFIAGTETVSTTLGYGFLLLMKHPEVEAKVHEEIDRV  
IGKNRQPKFEDRAKMPYMEAVIHEIQFGDVI PMSLARRVKKDTKFRDFFLPKGIEVF  
PMLGSVLRDLRFFSNPRDFNPQHFLGKQFKKRDAFVPFSIRKRNCFGEGLARMEI.F  
LFFTVMQNFRLLKSSQSPKIDIDVSPKHVGFATIPRNYTMSFLPR

791..970

exon

/gene=CYP2A6V2:

/number=1

1237..1399

exon

/gene=CYP2A6V2:

/number=2

2115..2264

exon

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## FIG.2A CONT.

exon	/gene=CYP2A6V2: /number=3 2499..2659 /gene=CYP2A6V2: /number=4 3207..3383 /gene=CYP2A6V2: /number=5 4256..4398 /gene=CYP2A6V2: /number=6 4873..5060 /gene=CYP2A6V2: /number=7 5577..5718 /gene=CYP2A6V2: /number=8 6308..6489 /gene=CYP2A6V2: /number=9 6490..6744	1646 a	2196 c	1746 g	1627 t
exon					
exon					
exon					
exon					
exon					
3'UTR					
BASE COUNT					
ORIGIN					

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555050" T584T250

## FIG.2A CONT.

BASE	COUNT	1	61	121	181	241	301	361	421	481	541	601	661	721	781	841	901	961	1021	1081	1141	1201	1261
aagttccct	gaaatatg	tctggtcttc	ctcccccttc	caatgaagaa	gatggcagtg	caatgaagaa	gatggcagtg	caatgaagaa	gatggcagtg	caatgaagaa	gatggcagtg	caatgaagaa	gatggcagtg	caatgaagaa	gatggcagtg	caatgaagaa	gatggcagtg	caatgaagaa	gatggcagtg	caatgaagaa	gatggcagtg	caatgaagaa	gatggcagtg
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ttcctgagac	ccttaacct	gcatactcca	caacagaaga	ccccctgga	acccccagat	ccccctgga	acccccagat	ccccctgga	acccccagat	ccccctgga	acccccagat	ccccctgga	acccccagat	ccccctgga	acccccagat	ccccctgga	acccccagat	ccccctgga	acccccagat	ccccctgga	acccccagat	ccccctgga	acccccagat
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cacagccctg	cggcaccct	cctgaagtac	cacagattta	gtctggaggc	ccccctctctg	gtctggaggc	ccccctctctg	gtctggaggc	ccccctctctg	gtctggaggc	ccccctctctg	gtctggaggc	ccccctctctg	gtctggaggc	ccccctctctg	gtctggaggc	ccccctctctg	gtctggaggc	ccccctctctg	gtctggaggc	ccccctctctg	gtctggaggc	ccccctctctg
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ctgggtgcga	agcattcccag	cacatgacat	ctcgtgtctg	ggcccccttc	agagtggagg	ggcccccttc	agagtggagg	ggcccccttc	agagtggagg	ggcccccttc	agagtggagg	ggcccccttc	agagtggagg	ggcccccttc	agagtggagg	ggcccccttc	agagtggagg	ggcccccttc	agagtggagg	ggcccccttc	agagtggagg	ggcccccttc	agagtggagg
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ttcaccattc	acttggggcc	ccggcgggtc	gtggtgctgt	gtggacatga	tgccgtcagg	gtggacatga	tgccgtcagg	gtggacatga	tgccgtcagg	gtggacatga	tgccgtcagg	gtggacatga	tgccgtcagg	gtggacatga	tgccgtcagg	gtggacatga	tgccgtcagg	gtggacatga	tgccgtcagg	gtggacatga	tgccgtcagg	gtggacatga	tgccgtcagg

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## FIG.2A CONT.

1321 gaggtctctgg tggaccaggc tgaggagttc agcgggcgag gcgagcaagc caccttcgac  
 1381 tgggtcttca aaggtatgg tgcccaagag ggggaaggtg ggcaggtgga cacgaaggtc  
 1441 tcagtgttcc cagccttctc cctgactctc ctgacaactg gaggataagg gagagtcccc  
 1501 agtctgggtct tccctcccca tctccctaca ttggggcctc tccatgtgta tccctcacct  
 1561 gtctccagcg gccctgtcct gattcctccc tgccctctctc tgccccacct ccttattctc  
 1621 tctcaactgga gtctcctctt tccctctctt tccctctctc ctccatctct tgggtttctg  
 1681 ttaccagcc ctgggtctct gtctacatga gtctttgagg ccctcttagc ttctgggclt  
 1741 ctctgggttt ctcatctctc cggatccctt tctcaattct tctctgtct taggatgcca  
 1801 gggttattcc tacttccaca tcttcaggct ccattctctg gtaacagtct ctcttctctc  
 1861 cagaccctct ctgtttctat ctcaatatca aactctctgc tccagctcag cttaagaatc  
 1921 tcacaccaag agaggatgtc ctccaccag atctcccat atctcactac ccacccctcc  
 1981 atcctctgcc tccatcactc tctttctctc cccactgccc tgcggacgag atccaatgga  
 2041 gtgtggagct aatgccgtga agctatgtgc atctctctgt ctggccgtac ctgggtaata  
 2101 acctgatacga ctaggcgtgg tattcagcaa cggggagcgc gccaaagcagc tctcgcgclt  
 2161 tgccatcgcc acctgaggg acttcggggc ttgcaagcga ggcatacgagg agcgcatcca  
 2221 ggaggagtcg ggcttctca tcgaggccat ccggagcacg cacggtgagc aggggacccc  
 2281 gagtgcgggg gcaggagaag gaaaacacc aggacgagga acccgcgcg gtctgcclg  
 2341 gggatgggga ctagggtggg aaaggcgccc gcacttccag ccctggagtc tggcgctggg  
 2401 aatttggctc aacaaggccc tgctcctgg aattctgact ctctcagac ctctgagttg  
 2461 actctctccc caacccctt ctccgacat acccgaggcc gccaatatcg atcccacct  
 2521 ctctctgagc cgcacagtct ccaatgtcat cagctccatt gtctttggg accgctttga  
 2581 ctataaggac aaagagtctc tgtcactgtt gcgcatgatg ctaggaaatct tccagltcac  
 2641 gtcaacctcc acggggcagg taatgggtgc agcccgccc gtgaaggccc ttaccaaac

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566060" TEST 260

## FIG.2A CONT.

2701 cggaattg tccccacc ggggaagg gggcccaat tccaccgc cccggacag  
 2761 tgtccctca aatcagtc cggattgg caattggca ggtggaacc agaccgggt  
 2821 tggttgtcca atccccgt ctccaggac accgggatag cacaacagat gctcccaaa  
 2881 acagagcctg ctggcaggat gcataccctc agtcagctc tctcaccctg ggcacgtgtt  
 2941 cccatcccca acttacgggt aatttctaac agatgctccc taccaggctc ttcttgaata  
 3001 ttttaacacc cggaaccctt gggtacctaa ccttcctgt aaactttaga gattagtccc  
 3061 tatccggccc ctctgaaata cctaaccacc ggagaccaga tgcctttaac tcagtccctt  
 3121 ccttgctatg aaacaaatcc cattcccatc agctcctgcc ccgtgacagc tgccttcccc  
 3181 tccccatcct ctctctgcaa cccagctct atgagatgtt ctcttcgggt atgaacacc  
 3241 tggcaggacc gcagcaacag gcctttcagt tgcgtcaagg gctggaggac ttcatagcca  
 3301 agaaggtgga gcacaaccag cgcacgctgg atcccaatc cccacgggac ttcattgact  
 3361 cctttctcat ccgcatgcag gaggtacacc ccagcagcca ctgcggggag atgcaaaagg  
 341 aggcagaggg aaatcagttt gggagtgggg caggcagatg acacaggccc attcaaatla  
 3481 accctcatca taataatcct cacaattggc tgggtgccgt ggctaacagc ctgtaatccc  
 3541 agcactttgg gagcccgagg caggtggatc acctgaggtc aggagttcga gaccagcctg  
 3601 gccaacatgg tcaaaccccc tctctactaa aaatccaaaa attagtggg catggtggcg  
 3661 cgaagggggg cagaggttgc aatgagccaa gatcacggca ttgcactcca gtctgggtga  
 3721 cagaatgagg ccctgtgtca aaaaaatta atcacttgtt taataaagtaa gtgagcctgc  
 3781 atggtcatgc gcatgtgcag ctccagctac tcaggaggct gaggtggag gattgcttga  
 3841 gctcaggagt tggcgtccgg cctgtgcaac ttagcaagac caagtcagta taagaaaaaa  
 3901 aaaaaacaaa aaaaagctg acagctaagt tgataattga cggacagatg gtcagcaagg  
 3961 taacgaagg gagagggaag agcatgggg gcaacgccag gattcagggc aagggtggt

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66000" TSBH260

## FIG.2A CONT.

4021 tcctagagcg agtctggtag gatctagggc cccttcttc caccctgcgg tcttgcccca  
 4081 aagagaggtc gaggtgctg ggattgcgct agactcgagt ctgttagat cttgggggtcc  
 4141 cctcttgacc cccattggtc tgaacctaa agtggaagat ccatgggggtg aacccttaga  
 4201 tggcgccctg aggtcaagca ggagtgaagt tgcctaaag cccctctcc cttcaggagg  
 4261 agaagaacc caacacggag tctactga agaacctgat gatgagcacg ttgaacctct  
 4321 tcattgcagg caccgagacg gtcagacca cctgcacta tggcttctta ctgctcatga  
 4381 agcaccaga ggtggagggt aaggctggag gggacggaa gtggaggcc ccagaccctc  
 4441 aaattcccc ttcgactggt gcaatgtccc cactgtccc agatccccgg accctgagac  
 4501 gtgacttgct gtccagagac agggcaacat tcagctggta ggcatcagct gagtctcatt  
 4561 agatattaaa atattgaaa tgtctgcact gattggtcag tcacttctgt cccaagccca  
 4621 ctgagtgcct actgccgtt ccaccgggtc atccccaa agtccccctg tgcctcccc  
 4681 gtgattctgg cacaacctgg ttaacaggat cctactccaa caatgcgaat gggtgatgtc  
 4741 tgttctgtta tgaatgctct acttccgtct cataggcga ggcatttcat ccaccccat  
 4801 ttgcctatcc ggactatcat ttcctgctct gagacccta gatacctaaa cacattcccc  
 4861 ctccctcccc agccaaggct catgaggaga ttgacagagt gatcggaag aaccggcagc  
 4921 ccaagtttga ggaccgggccc aagatgccct acatggaggc agtgatccac gagatccaaa  
 4981 gatttggaga cgtgatcccc atgagtltgg ccgcagagt caaaaaggac accaagtctc  
 5041 gggatttctt cctccctaag gtgctatccg cccccacc ccagactacg gggactccag  
 5101 cccctctctg tgtcccccag atccccca cattagaagc tttctagacc ctgtccact  
 5161 ccctcaatca gtcaaaaaag acttcccaa ccaccacat cgttccacct ttccacttag  
 5221 aactcctga gtctgcatc tctccagact ctttgtgtca ggagaatcaa acacatgttc  
 5281 ccaacttcc tatcttaaga aacagaagcc ccttttccat tcggcctttt gtcataggga  
 5341 cagaaatctc aggtccccc aactcctgcc tagaaggaca tggaccccat gttcccaaa



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## FIG.2A CONT.

5401 cttcctgttt cagagatgtg aaccttctat ccccaaggt cctccctcag aggtcccaa  
5461 ttcccatgcc tgccacttcc cctcaccggg gcacctagt tccccctcca gcccctgtgt  
5521 actctcaaca atcccccaac ccgctcacc acatacact tctctctccc tcccaggga  
5581 tagaagtgtt ccctatgttg ggctccgtgc tgagagacct caggttcttc tccaaccccc  
5641 gggacttcaa tccccagcac ttctgggtg agaaggga gtttaagaag cgtgatgttt  
5701 ttgtgccctt ctccatcagt aagagaccac tgtttgggtc caggcttact actcacacca  
5761 gcaggggcct cctttaccca gtccccctct ctgccgtgta gcctagtatt tccccagctt  
5821 ggcaagtccc tgttagcaat ctaccgtga gccaccaggt gatactccct taactaccaa  
5881 gcaccagta cctgtgccc aagaaagga aagaaacat cataccctt tcagaggcgg  
5941 gggaaaacca aaggccagag agaatacagag atttatttcc ctagggtcac acaggagatt  
6001 cttcagcatc cctaaaaagg agatgacggc acagcaggtc atatttggga gttcttatct  
6061 gggggaaggg ggtcttaaa cctcccattg tggacacctg gcacgatca accccatctt  
6121 ttggtcatct ttgggtcac tcaaggaaac tgaggtcaag gaggtcaag aggtccctc  
6181 ttaaagtctc tcagggccat atattccacc ctctctcctt gggagagccg cagctggagg  
6241 tcggtactgg ggcgaggctg cactgagagt gggttcacc tccacccctc ccgctctctc  
6301 tcctcaggaa agcggaactg ttctcgagaa ggcctggcca gaatggagct cttctctctc  
6361 ttcaccaccg tcatgcagaa ctctcgctc aagtcctccc agtcacctaa ggacattgac  
6421 gtgtccccc aacacgtggg ctttgccacg atccccgaa actacaccat gagetctctg  
6481 ccccgctgag cgaggctgt gccggtgaag gctggtggg cggggccagg gaaagggcag  
6541 ggccaagacc gggcttggga gaggggcgca gctaaagactg ggggcaggat ggcggaaaag  
6601 aaggggcgtg gtggctagag ggaagagaag aaacagaagc ggctcagttc acctlgataa  
6661 ggtgcttccg agctgggatg agaggaagga aaccttaca ttatgctatg aagagtagta

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565060" TSBH7260

## FIG.2A CONT.

6721 ataatagcag ctcttatttc ctgagcacgt acccccggtg cacctttgtt caaaaacccat  
6781 tgcacgctca cctaatttgc cacaaaaccc ccttcgaagg ggcgttcattg cccattttac  
6841 acgtgacaaa actgaggctt agaaagtgtg ctctgatgtc tcacaaaaca taagtgccca  
6901 gaaaatctgc gaacacagat ctgtgccccat agccttctag acagattctt aaaaagcacc  
6961 tattcctcac gcaaacacagt ttagtataga atcacatggc ctgaacatcc ctgtccgggg  
7021 gagttcccca gagacctggg ggggtggttc cctgccttca ctgcacacat gccacactc  
7081 tcacctactc aacatgctgt gactaccggg gtgtaatctg tgcttgctac cagataaggc  
7141 cactgtagcc cattcagagt cagcccaggg acacaacgag acatgactgg acatacaggg  
7201 tcagtccatt acaa

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# FIG.2B

LOCUS	HSP452B6	1415 bp	RNA	PRI	29-MAY-1992
DEFINITION	Human MRNA FOR CYTOCHROME P-450IIB6.				
ACCESSION	X13494				
NID	g35206				
KEYWORDS	Cytochrome; cytochrome P450IIB6.				
SOURCE	human.				
ORGANISM	Homo sapiens				
	Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;				
	Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.				
REFERENCE	1 (bases 1 to 1415)				
AUTHORS	Miles, J.S.				
TITLE	Direct Submission				
JOURNAL	Submitted (10-NOV-1988) Miles J.S., Imperial Cancer Research Fund,				
	Lab of Molecular Pharmacology and Drug Metabolism, Hugh Robson				
	Building, George Square, Edinburgh, EH8 9XD				
REFERENCE	2 (bases 1 to 1415)				
AUTHORS	Miles, J.S., McLaren, A.Q. and Wolf, C.R.				
TITLE	Alternative splicing in the human cytochrome P450IIB6 gene				
JOURNAL	generates a high level of aberrant messages				
MEDLINE	Nucleic Acids Res. 17 (20), 8241-8255 (1989)				
COMMENT	90045947				
	The sequence is a compilation of genomic and cDNA clones. **map:				
	chromosomal location=19q12-13.2;				
	Data kindly reviewed (13-NOV-1989) by Miles, J.S.				
FEATURES	Location/Qualifiers				

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09/214851

566050" T584F260

## FIG.2B CONT.

```

source      1..1415
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misc-feature 585..761
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misc-feature 762..903
             /note=exon 6"
misc-feature 904..1091
             /note=exon 7"
misc-feature 1092..1233
             /note=exon 8"
misc-feature 1234..1415
             /note=exon 9", coding region"
BASE COUNT   341 a   430 c   328 g   316 t
ORIGIN
1 gaattccgcc ctgcacccat gaccgcctcc caccagggcc cggccctctg ccccttttgg

```

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555050" T584T250

## FIG.2B CONT.

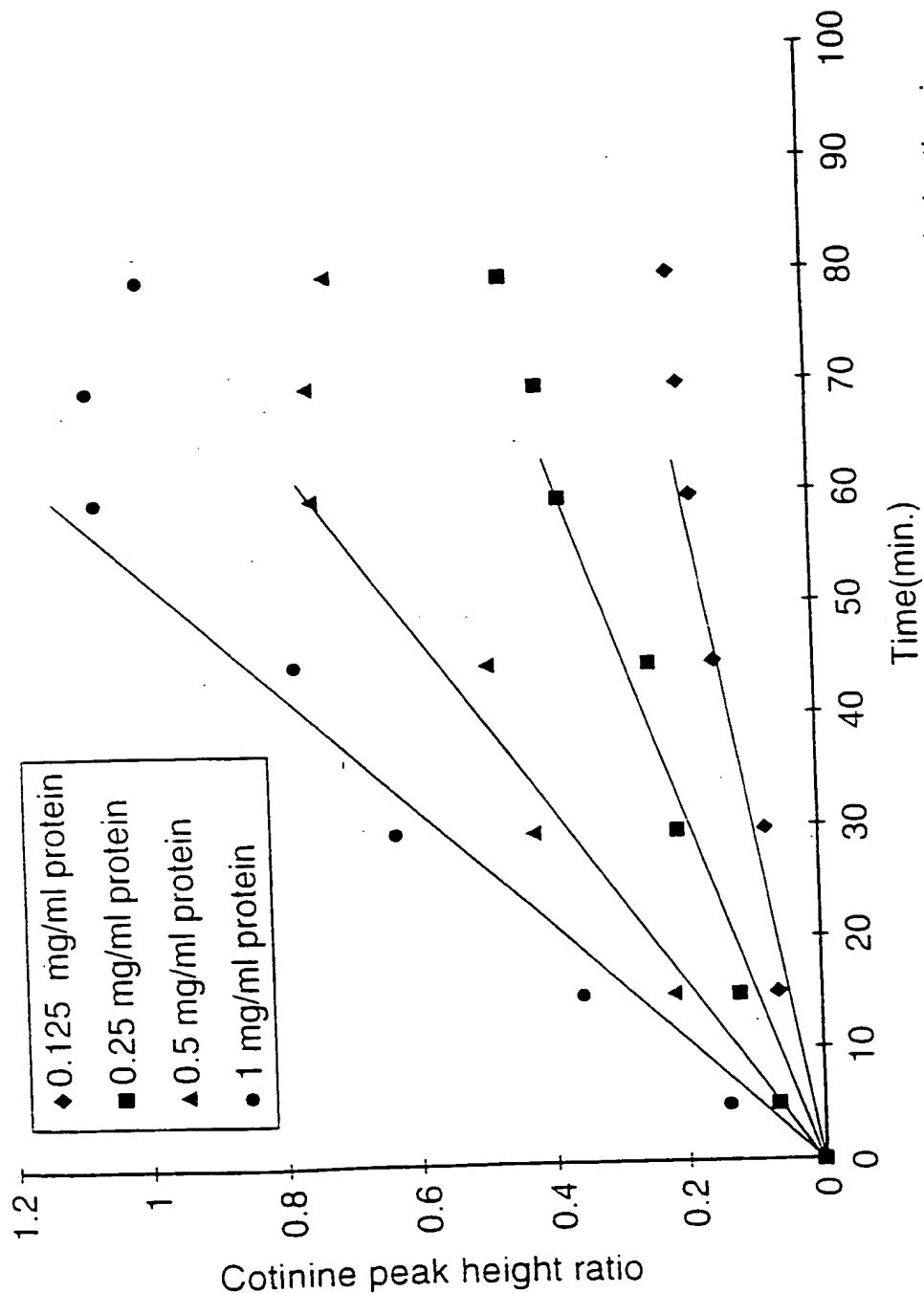
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361 gtgtggagga gcggaattcag gaggaggctc agtgtctgat agaggagctt cgaaatcca
421 agggggccct catggacccc accttctct tccagtccat taccgccaac atcatctgct
481 ccatacgtctt tggaaaacga ttccactacc aagatcaaga gtacctgaag atgctgaact
541 tgttctacca gactttttca ctcatcagct ctgtattcgg ccagctgttt gagctcttct
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781 gtgaattcag ccaccagaac ctcaacctca acacgctctc gctcttcttt gctggcactg
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1021 accttctccc catgggtgtg cccacattg tcaccaaca caccagcttc cgagggtaca
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1321 gccccgtggc ccagaagac atcgatctga cccccagga gtgtggtgtg ggcaaaatcac
1381 cccaacata ccagatccgc ttctgcccc gctga

```

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Protein-time curves of cotinine production from 100  $\mu$ M nicotine in the presence of 20  $\mu$ l rat cytosol by K20 human liver microsomes.

FIG.3

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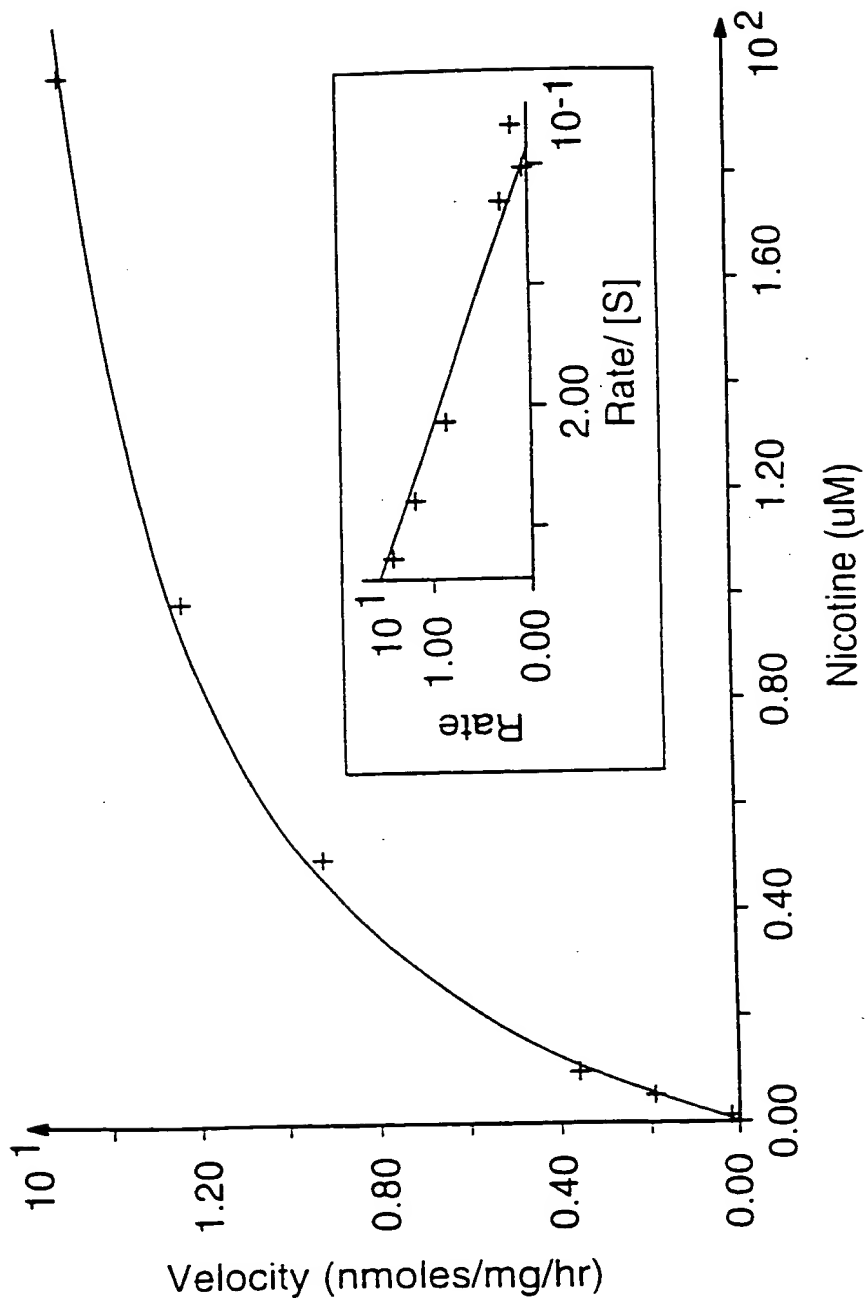


FIG. 4A

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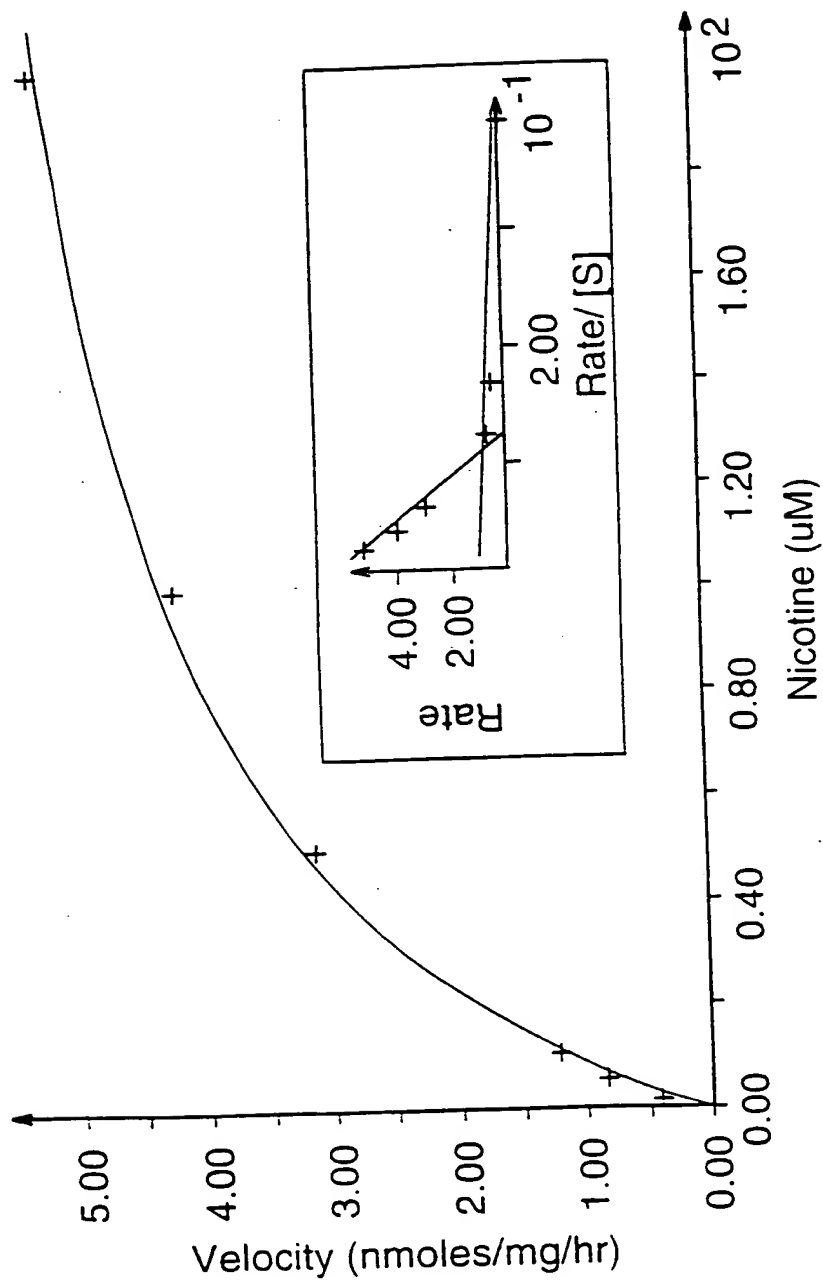
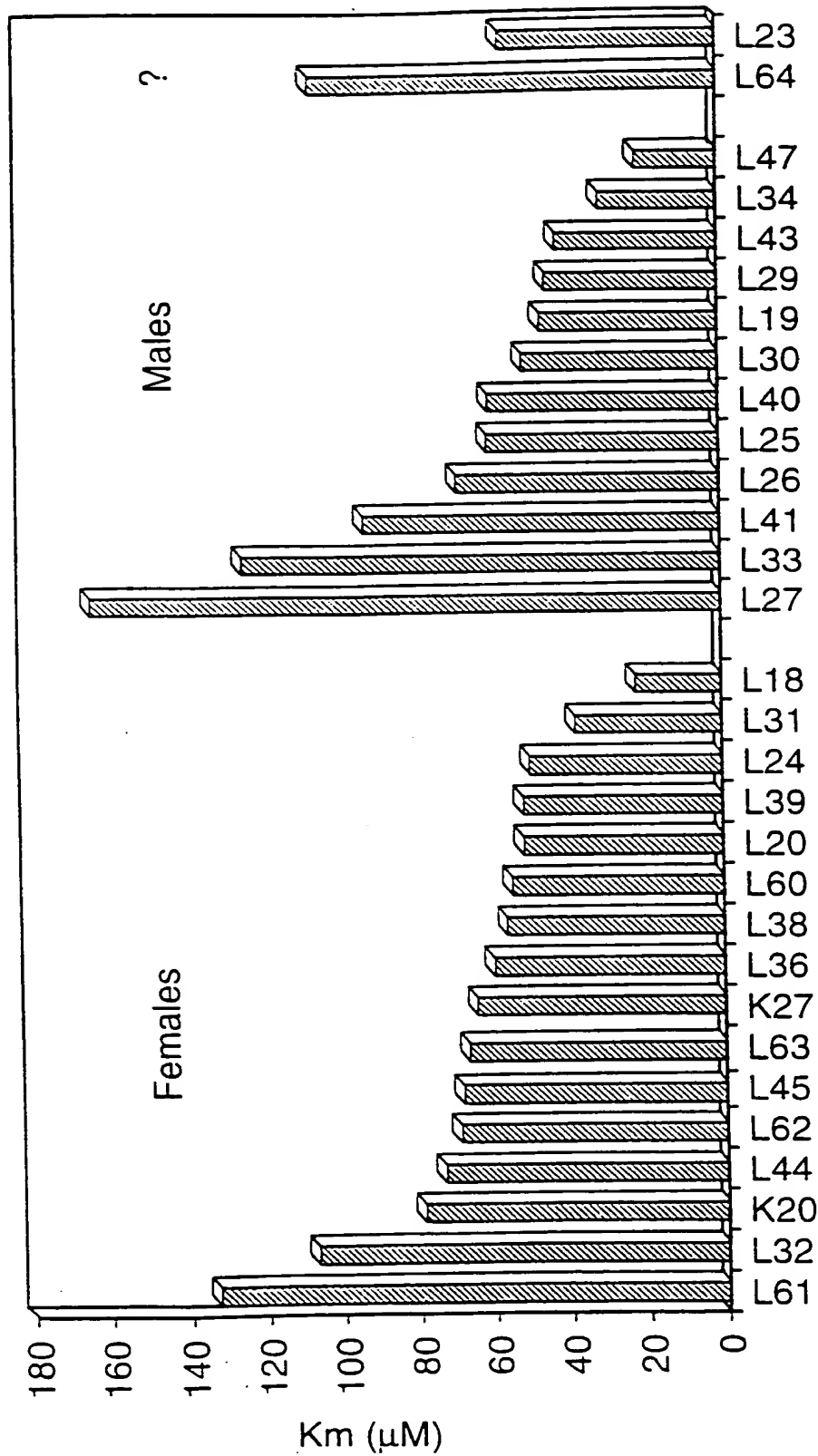


FIG. 4B



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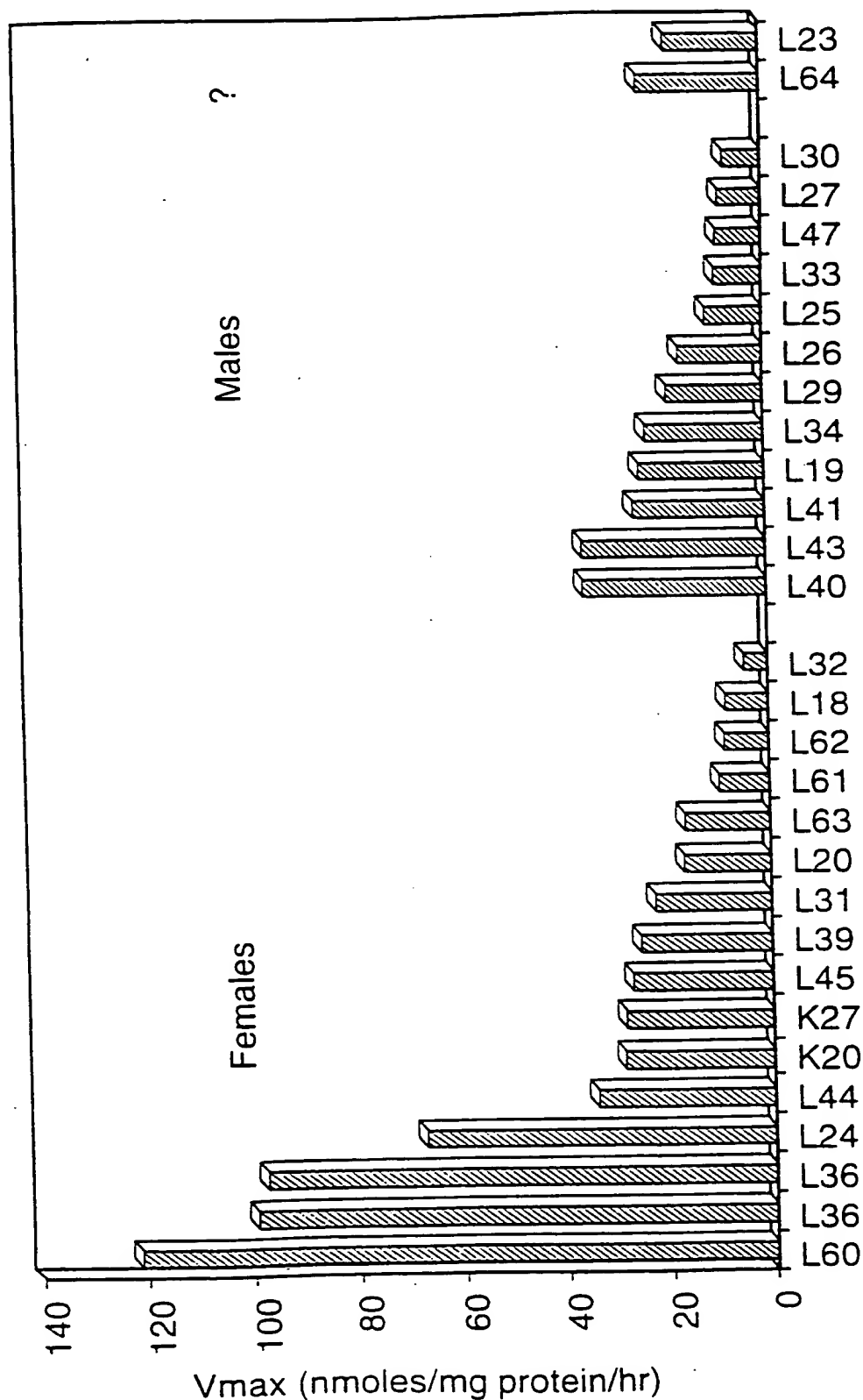


Liver Sample

FIG.5

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Liver Sample  
FIG.6

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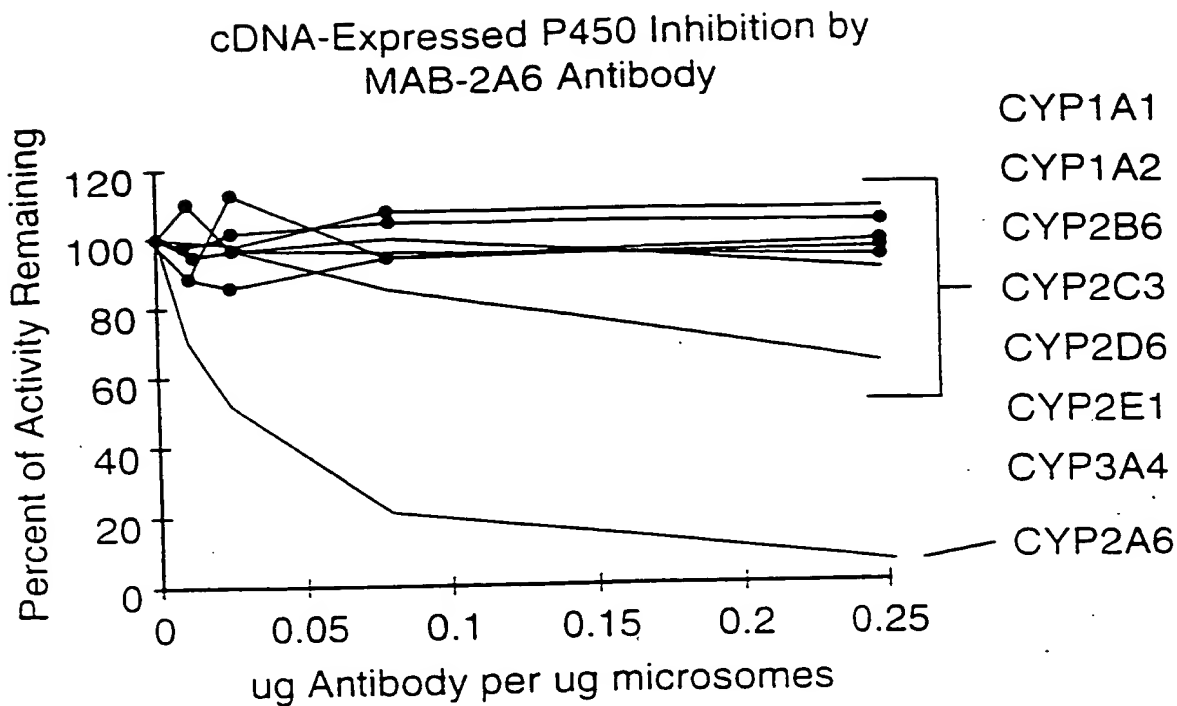


FIG.7A

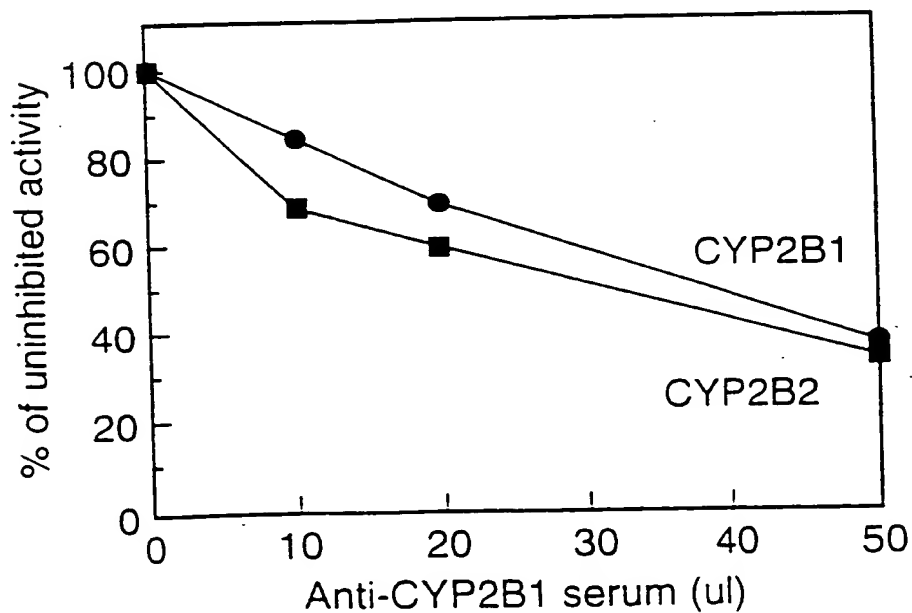


FIG.7B

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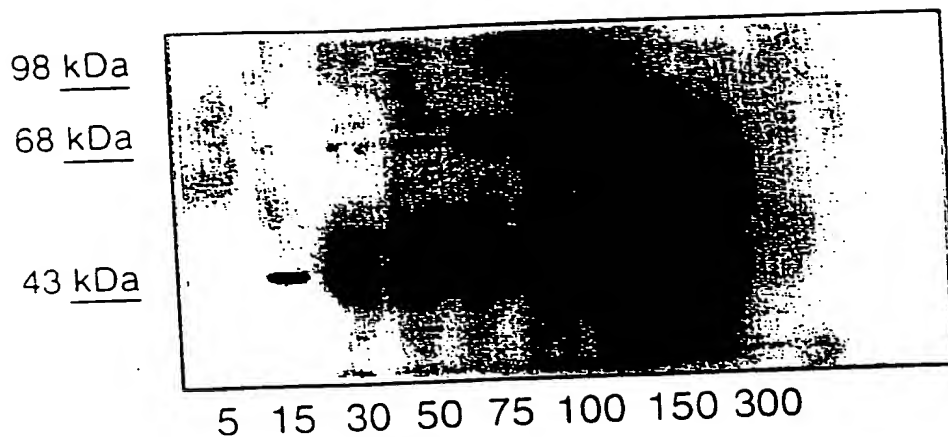


FIG.8A

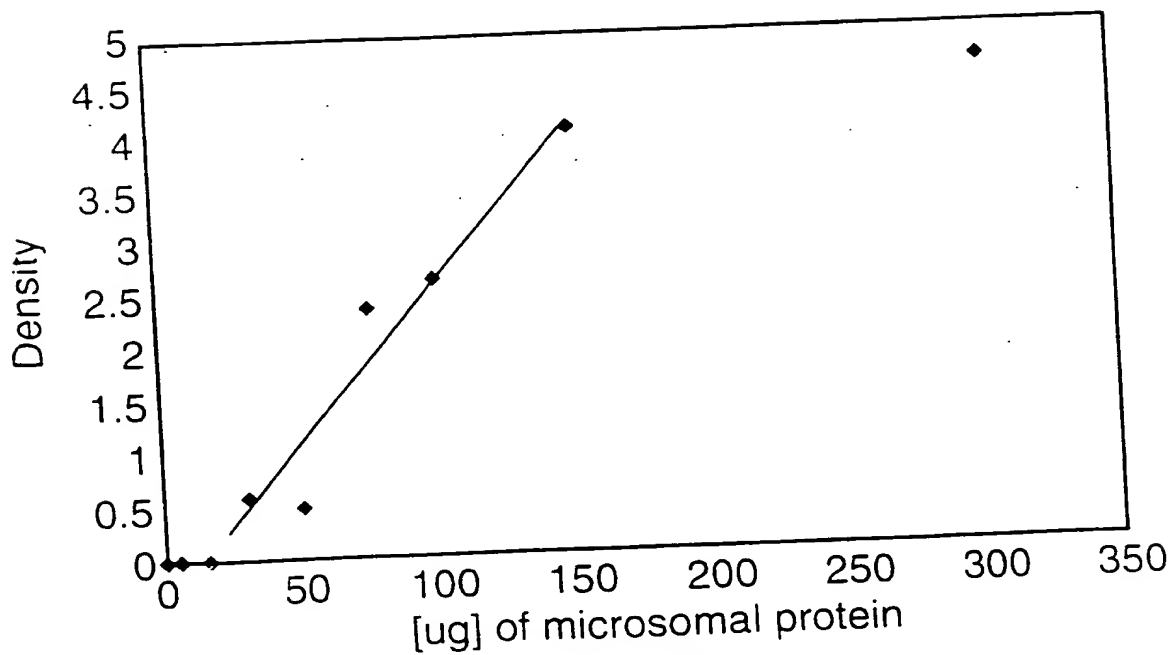
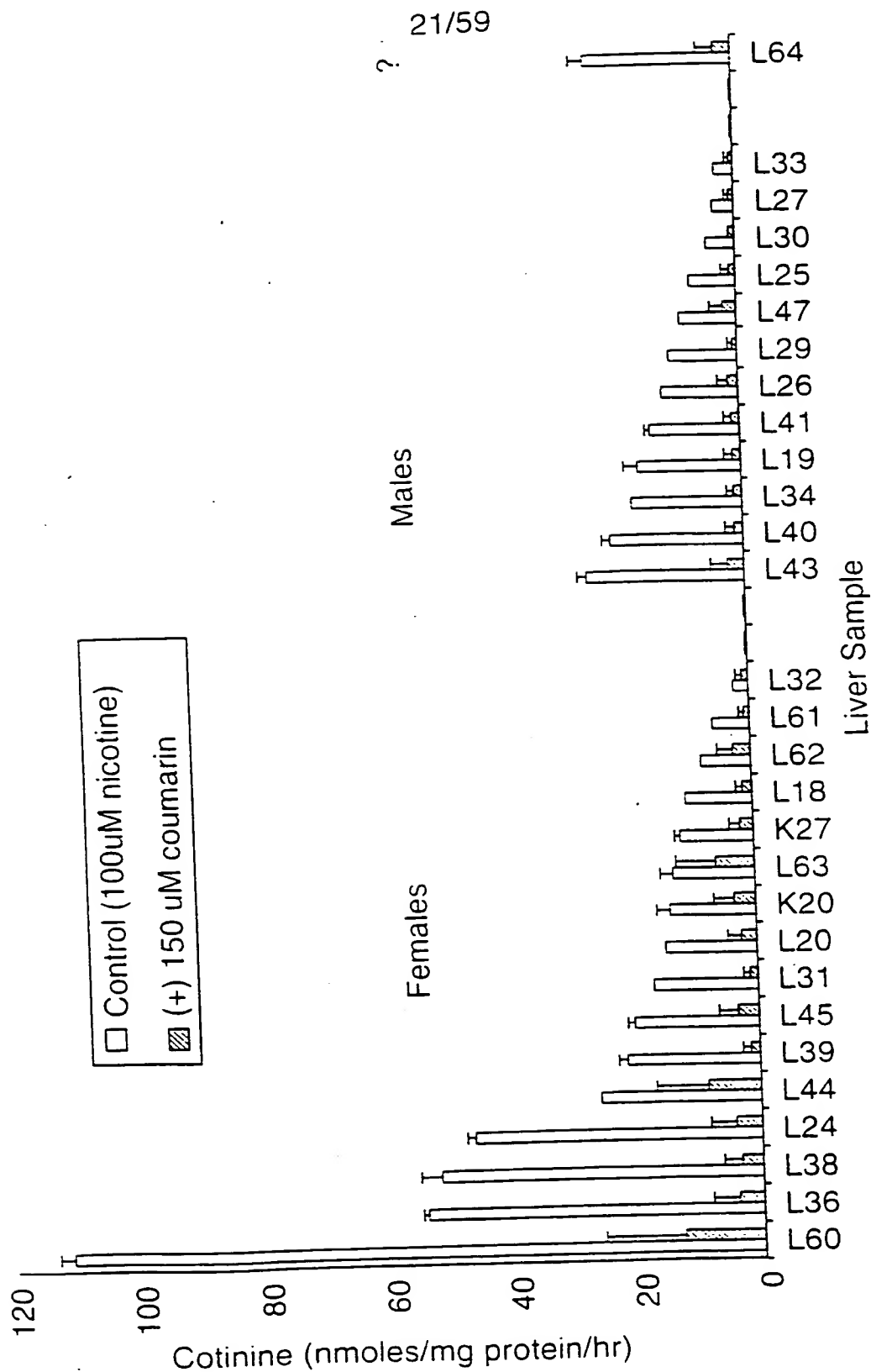
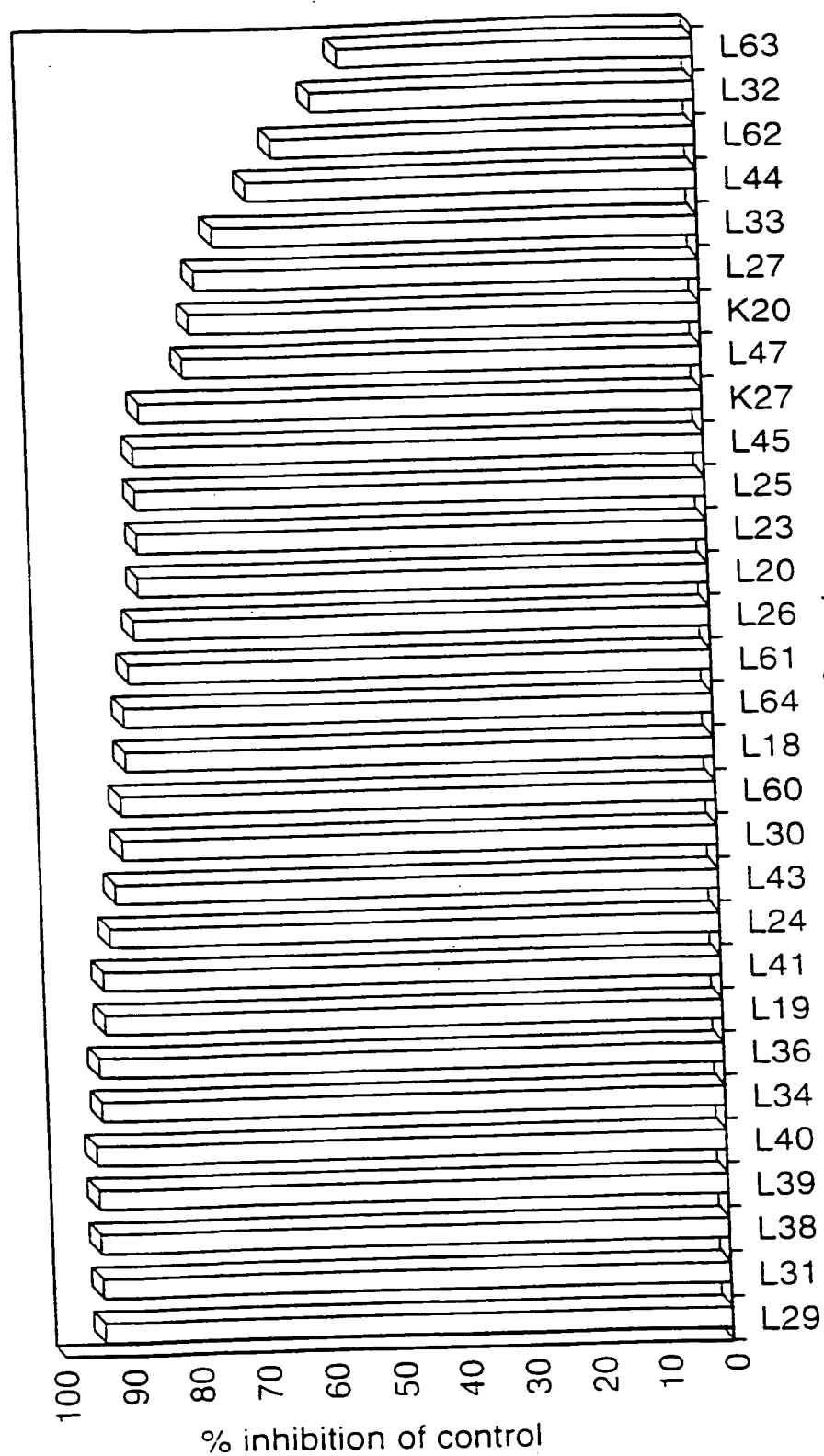


FIG.8B



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Liver Sample  
FIG.10

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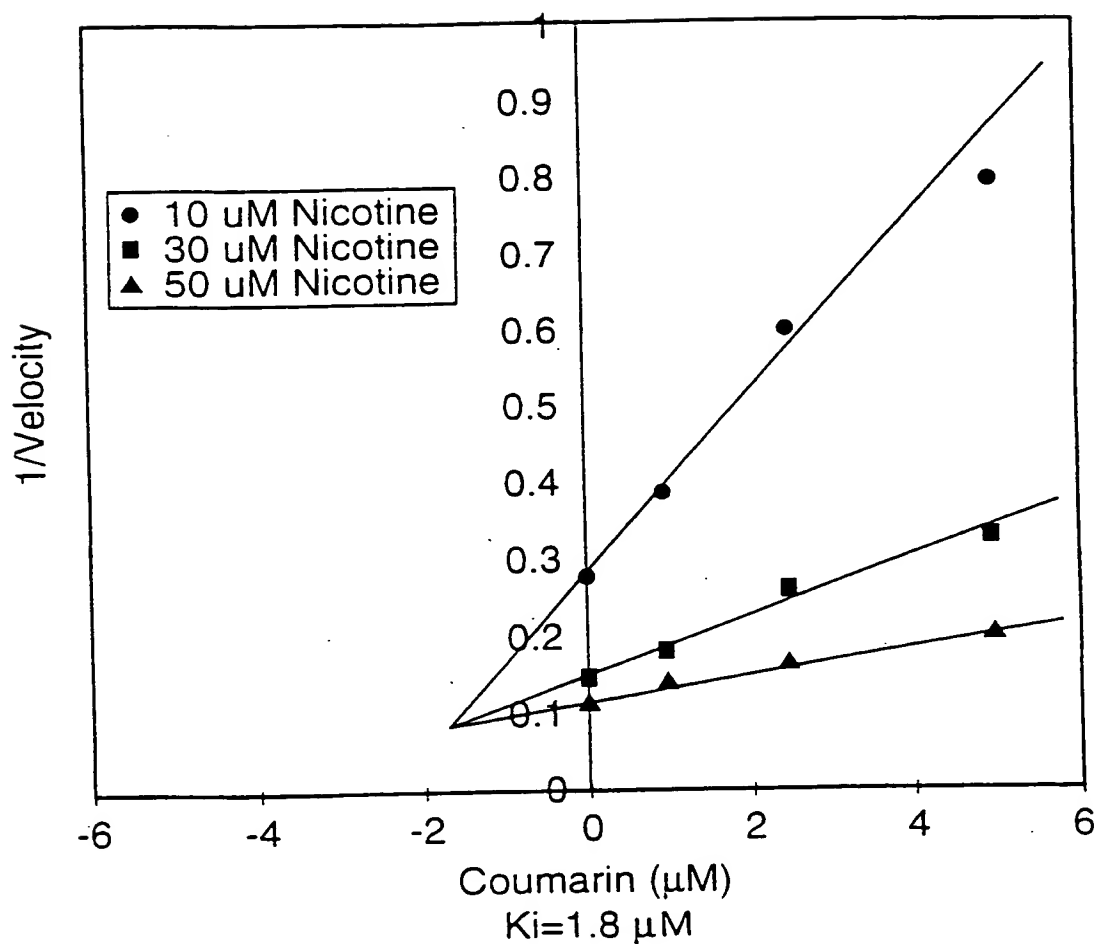


FIG.11

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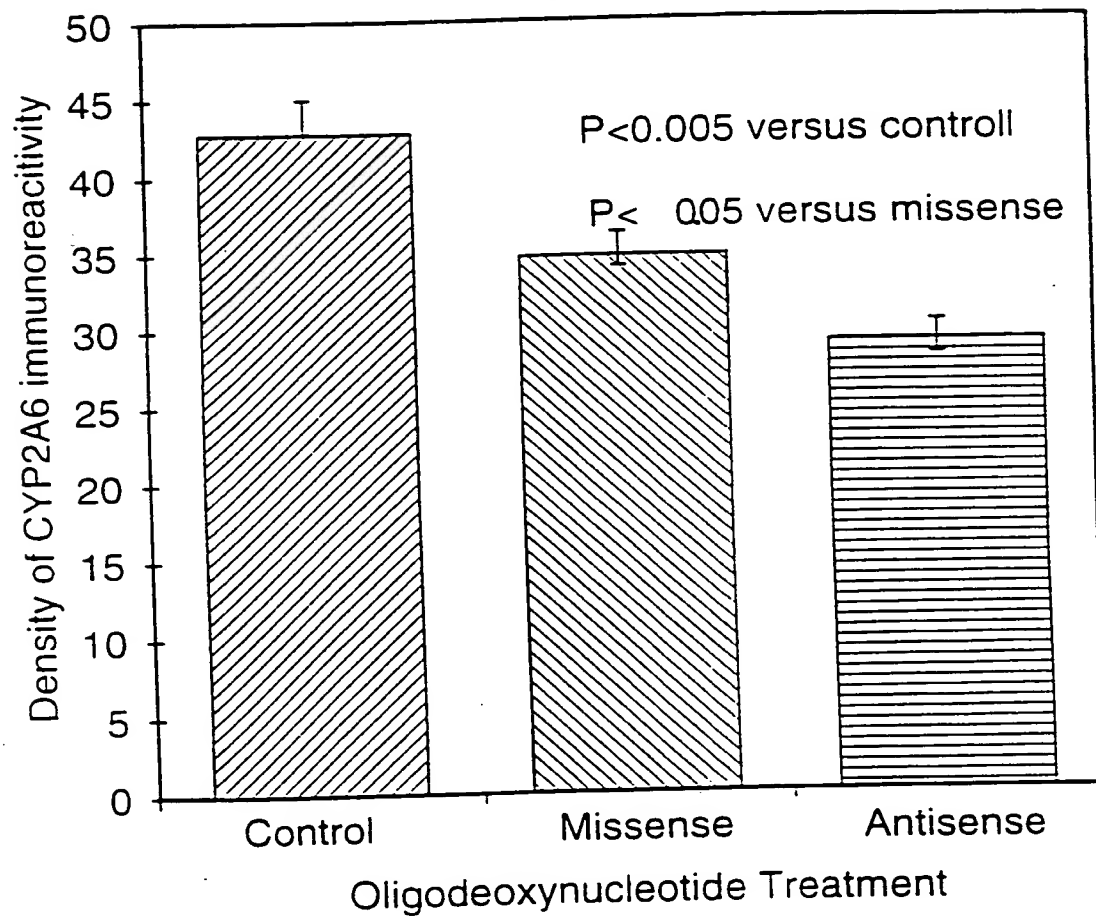
CYP2A6 Antisense Knockdown  
in HepG2 Cells

FIG.12



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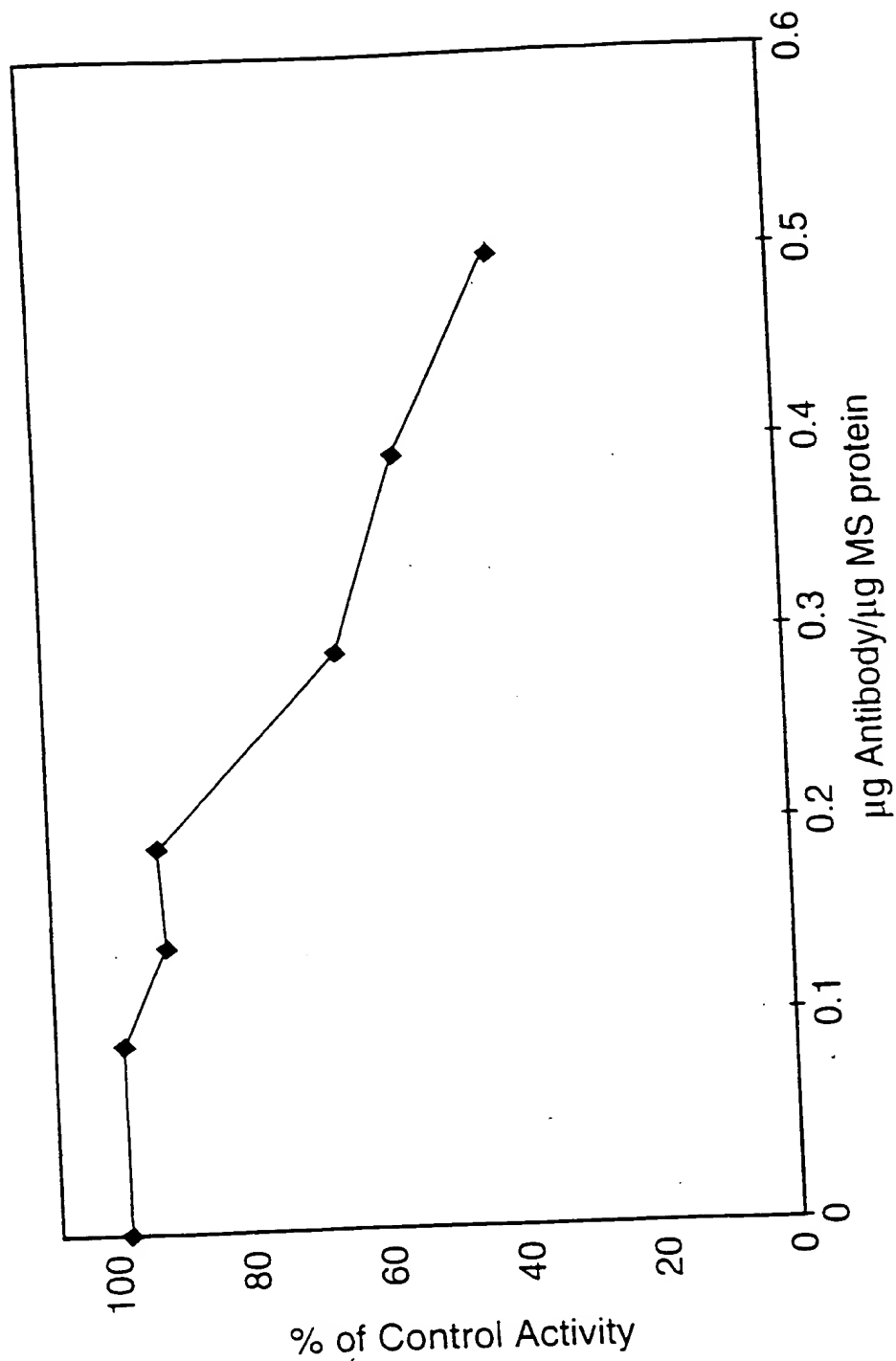
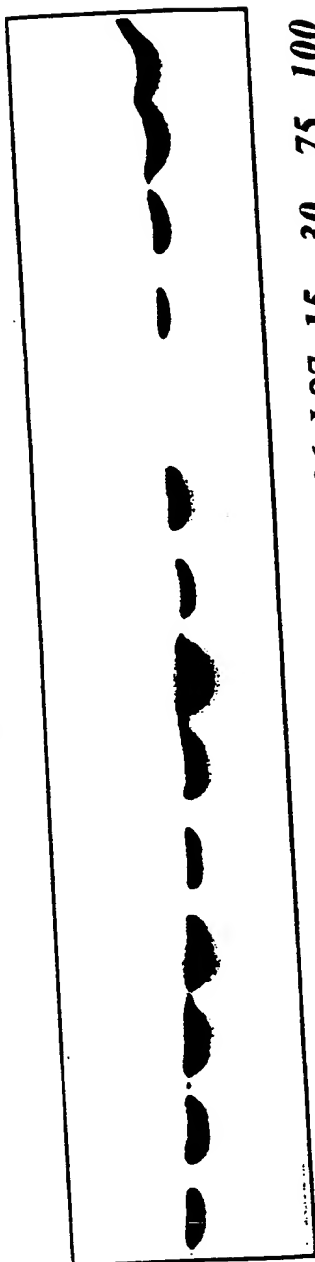


FIG.13

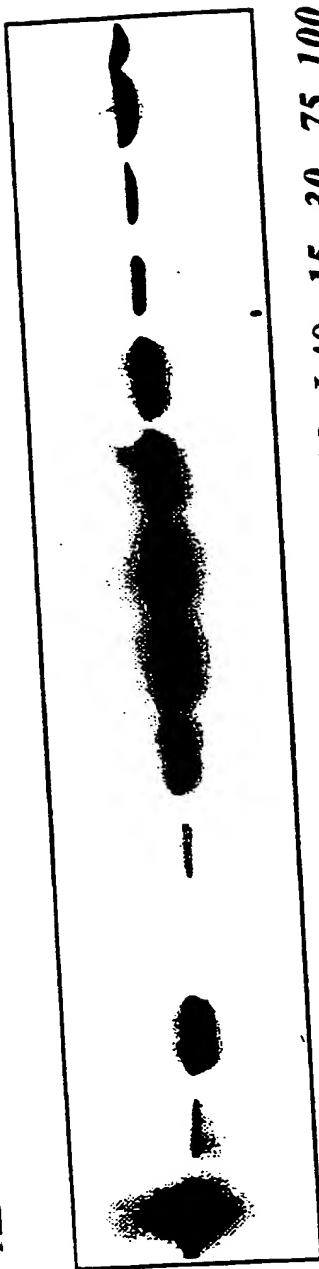
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FIG. 14A.



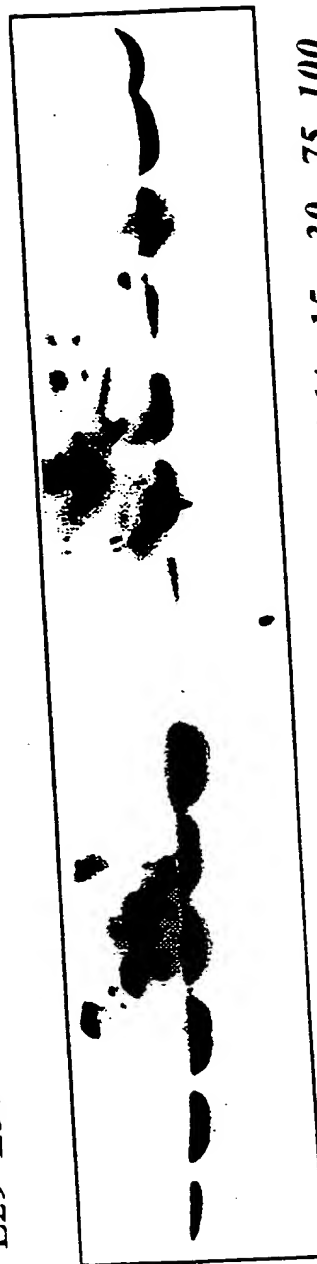
K20 L18 L19 L20 L23 L24 L25 L26 L27 L5 30 75 100

FIG. 14B.



L29 L30 L31 L32 L33 L34 L36 L38 L39 L40 L5 30 75 100

FIG. 14C.



L41 L43 L44 L45 L47 L60 L61 L62 L63 L64 L5 30 75 100

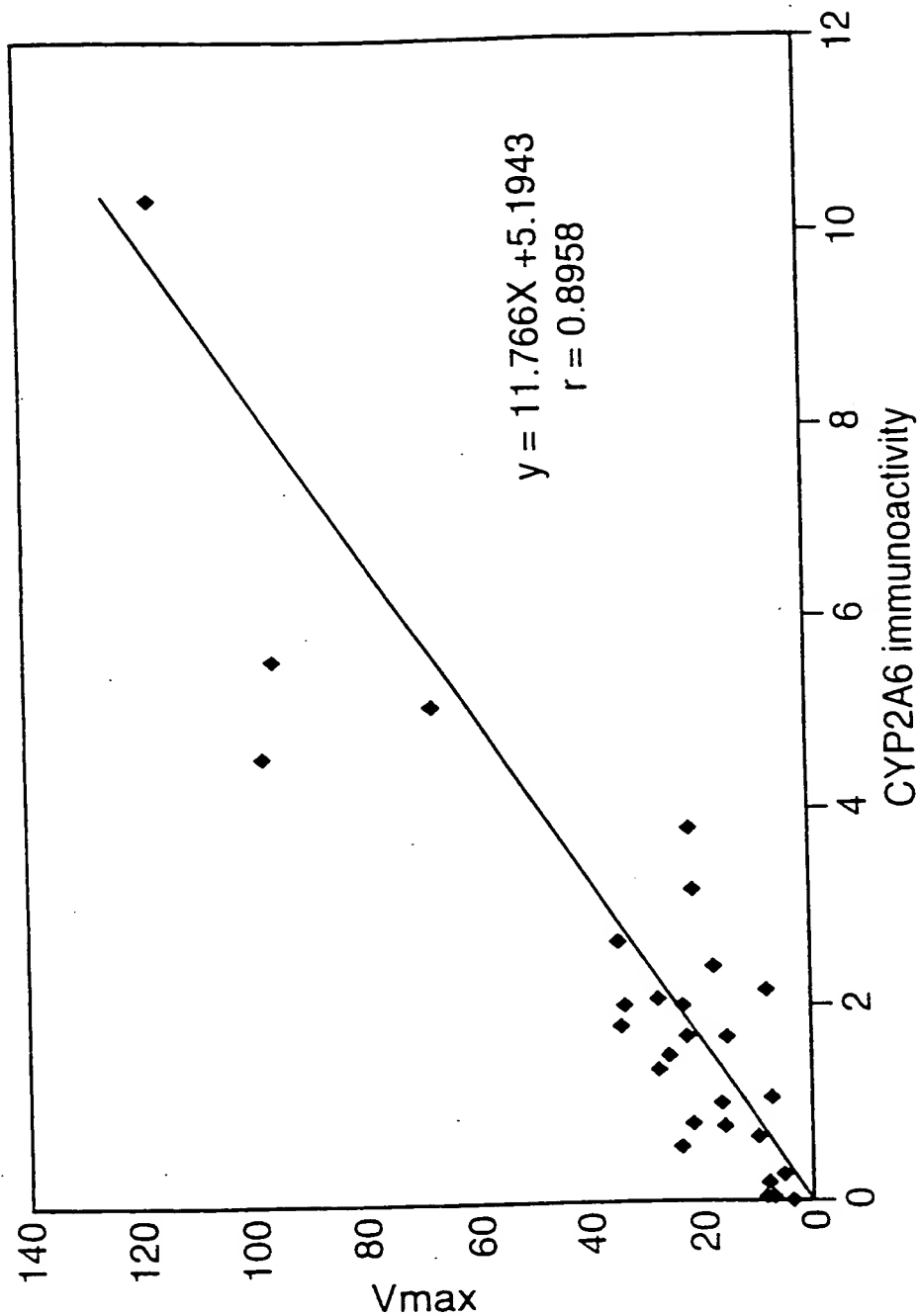
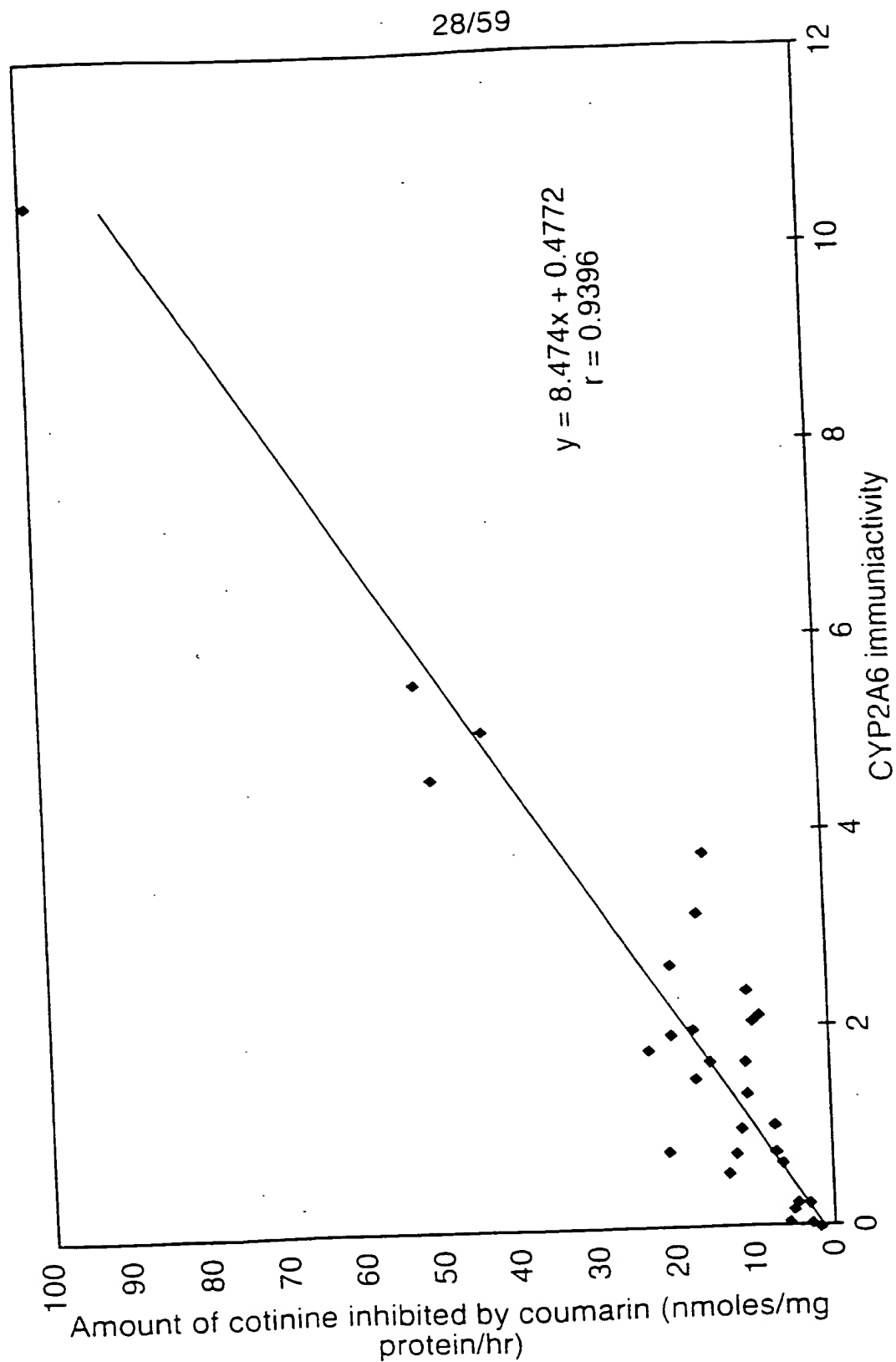


FIG.15

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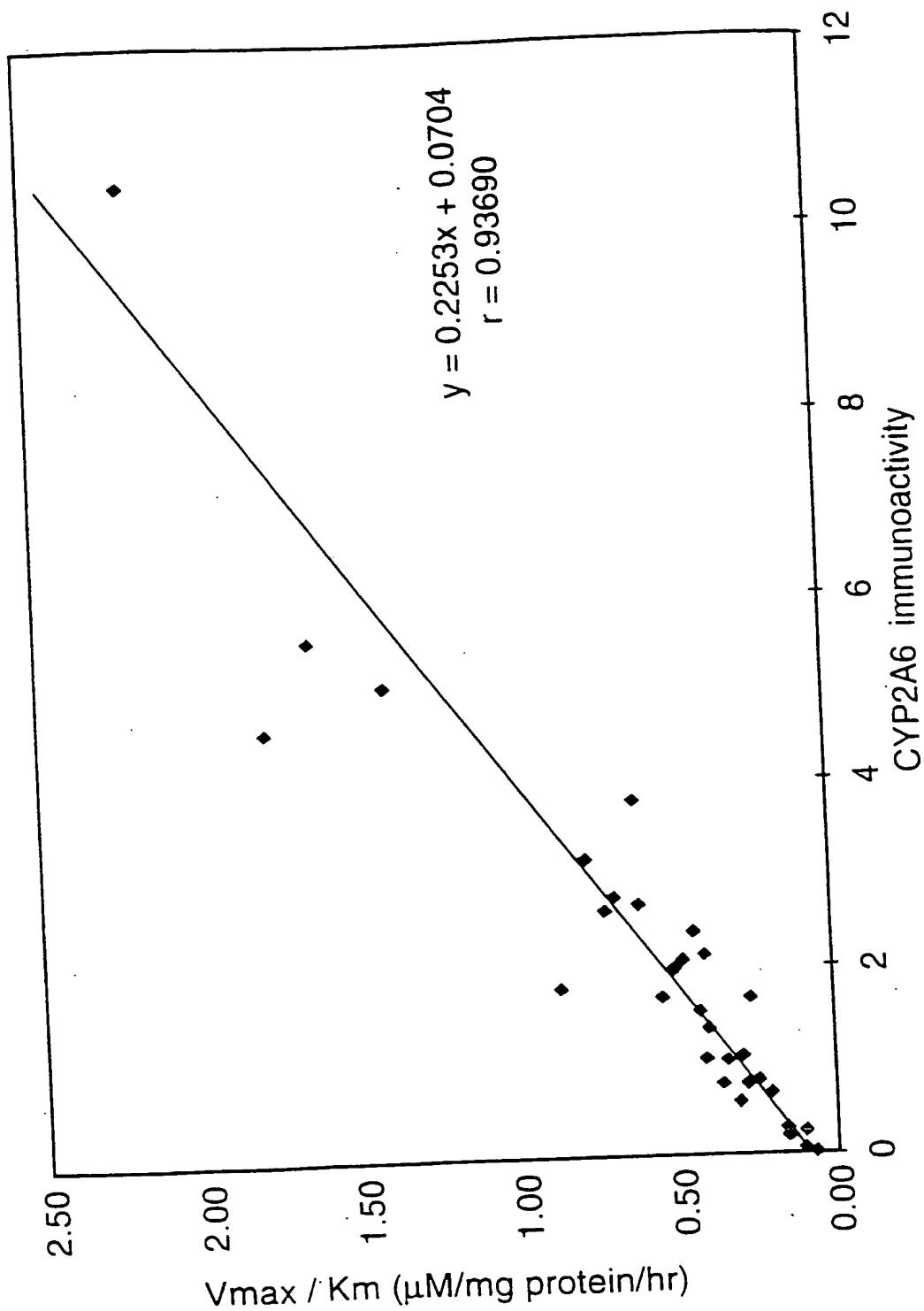


FIG.17

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?

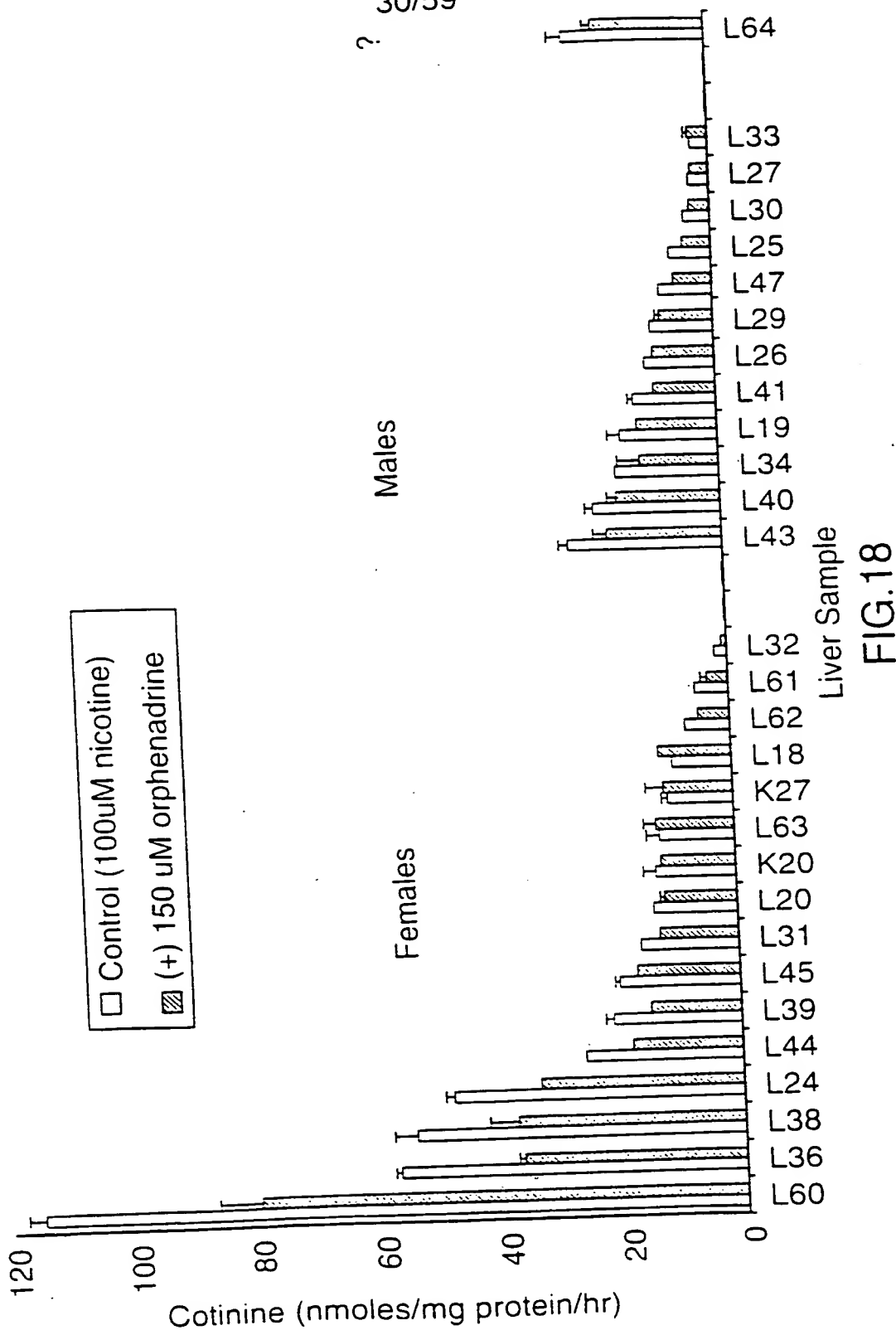
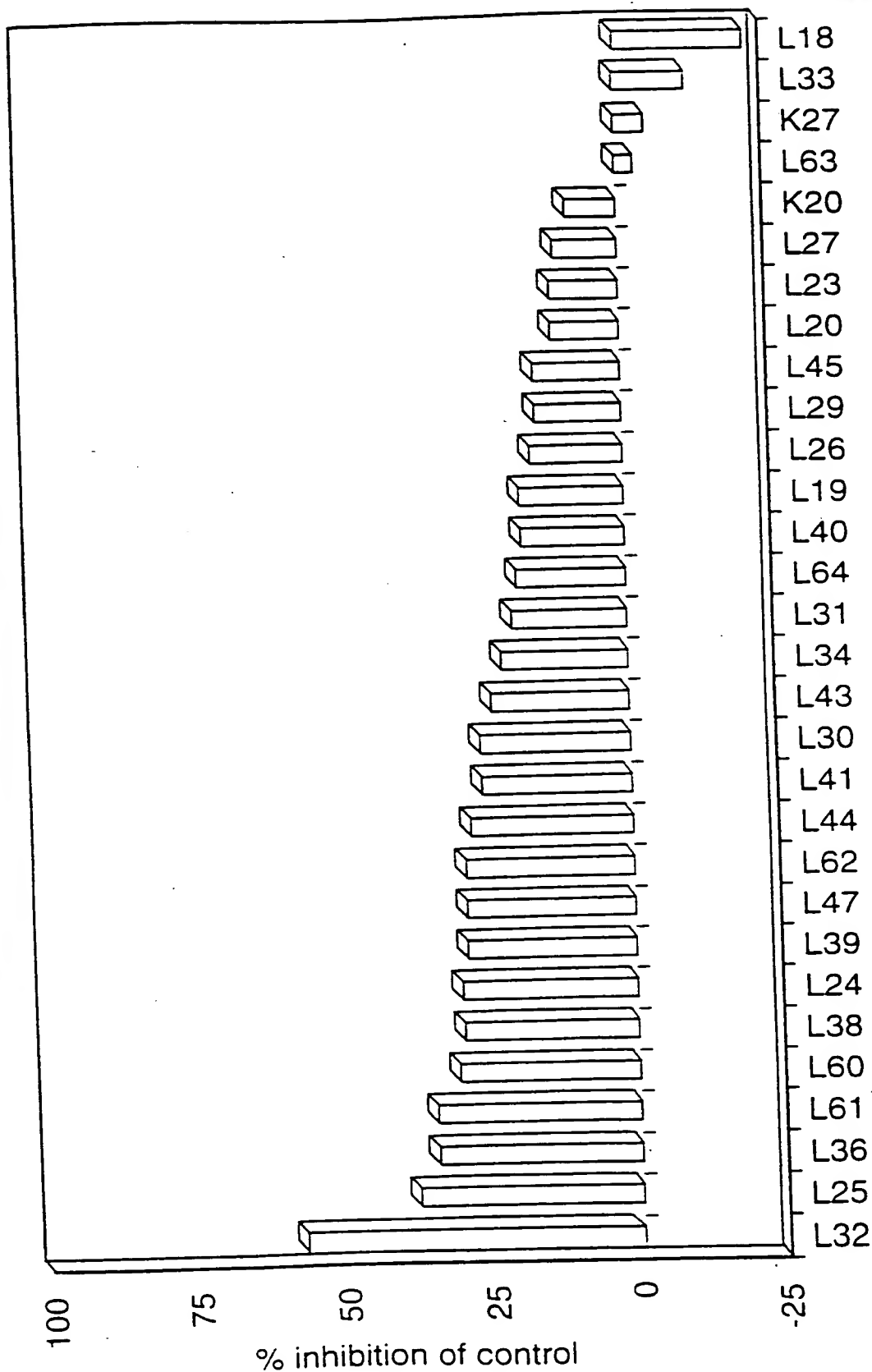


FIG.18

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Liver Sample

FIG.19

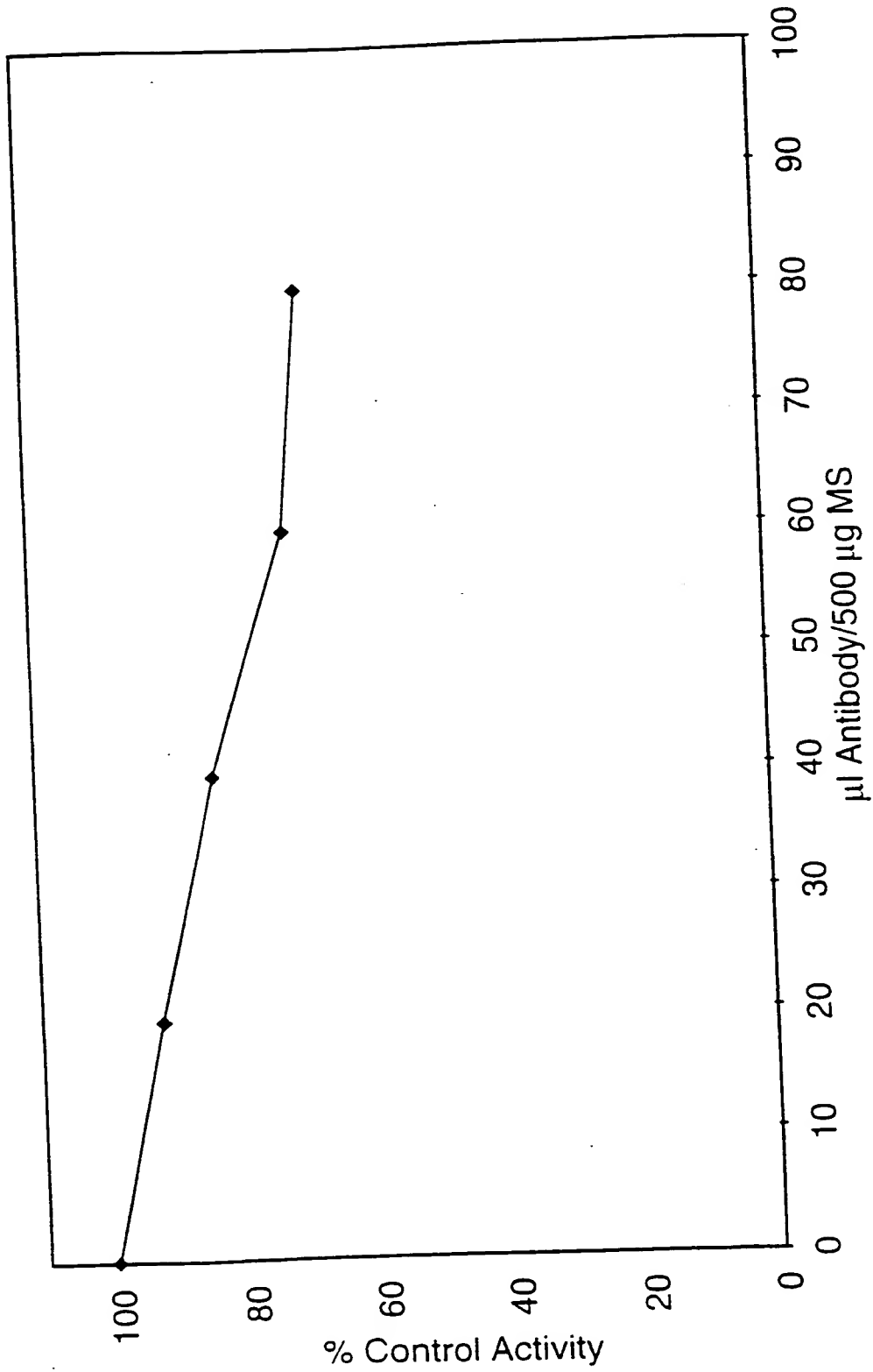


FIG.20



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?

Males

Females

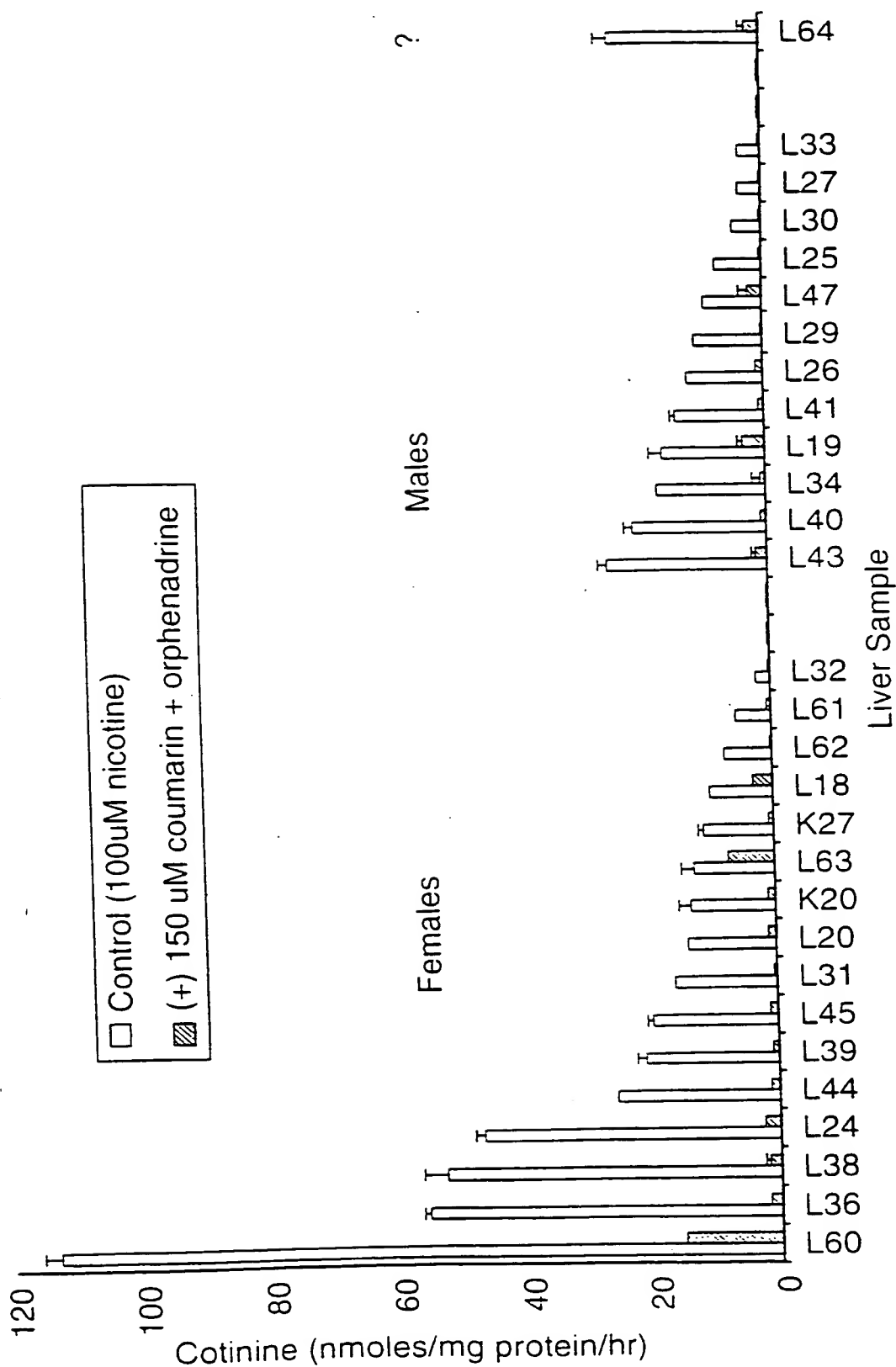


FIG.21

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?

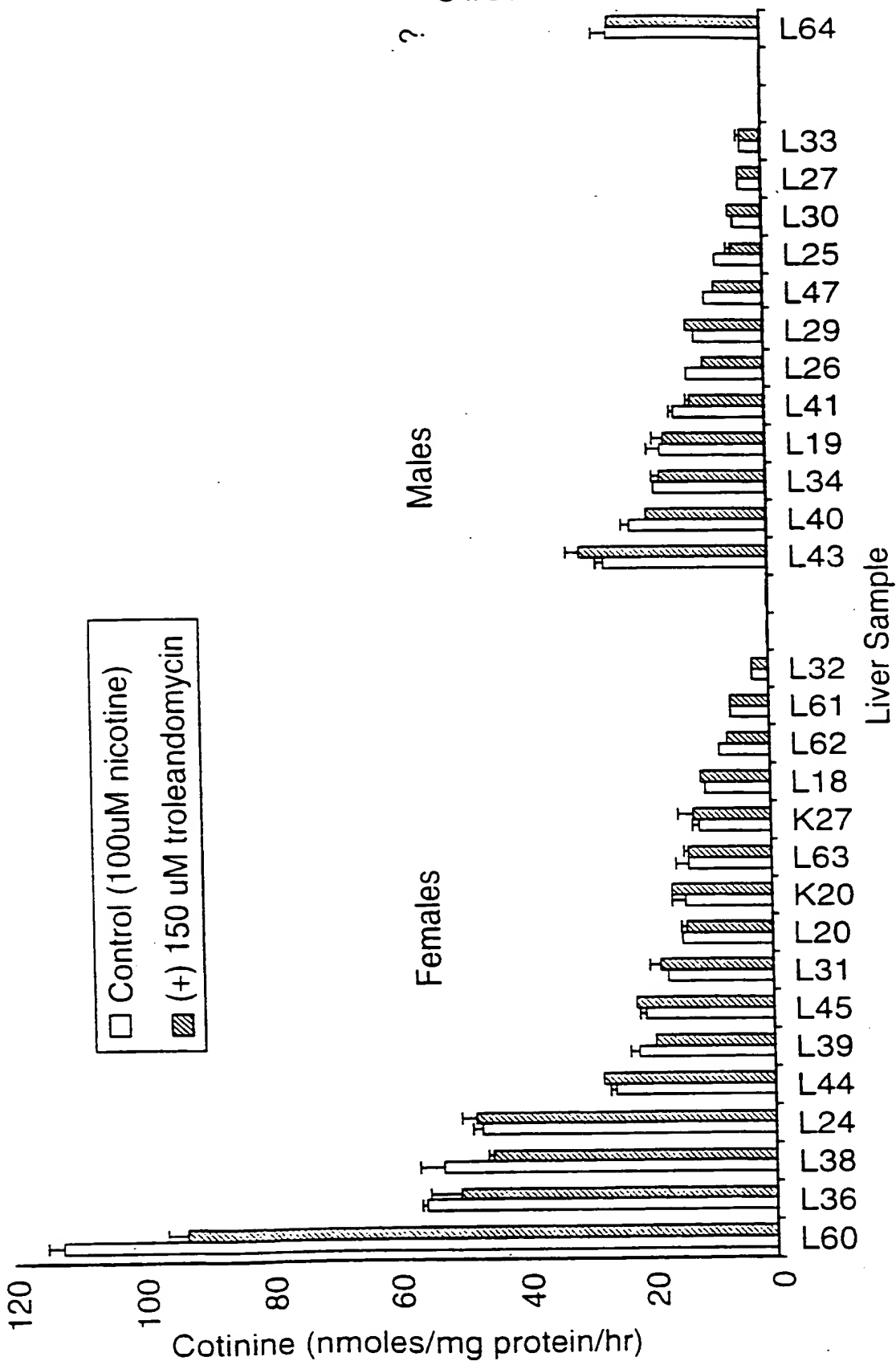


FIG.22

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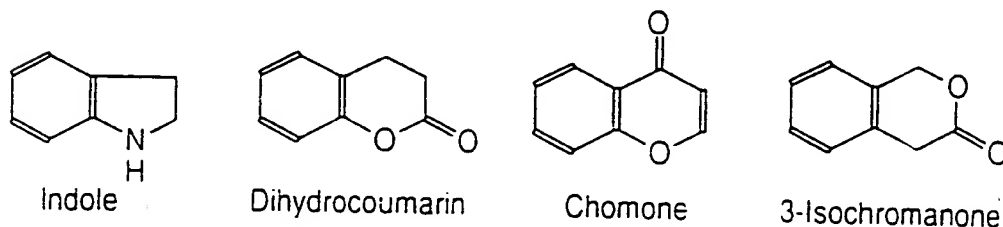
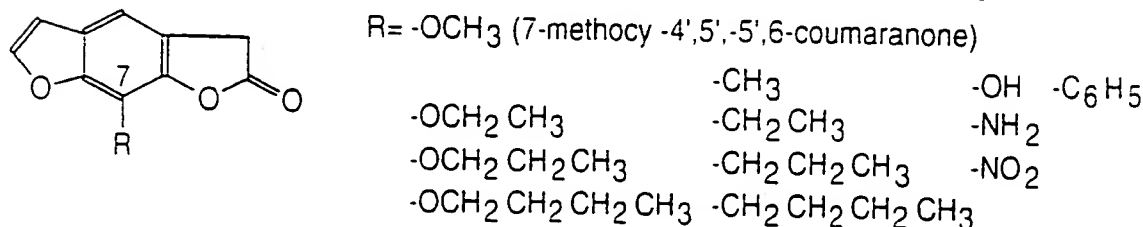
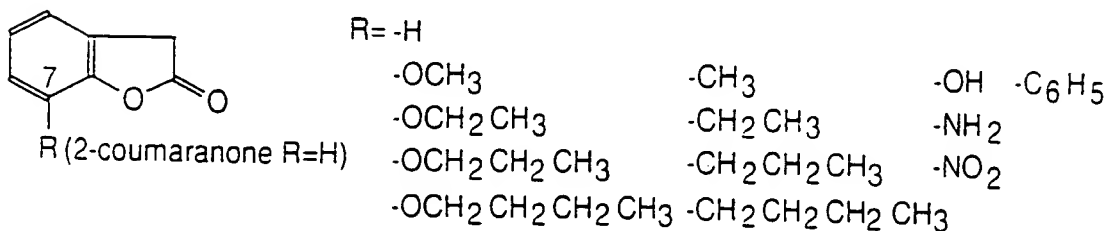
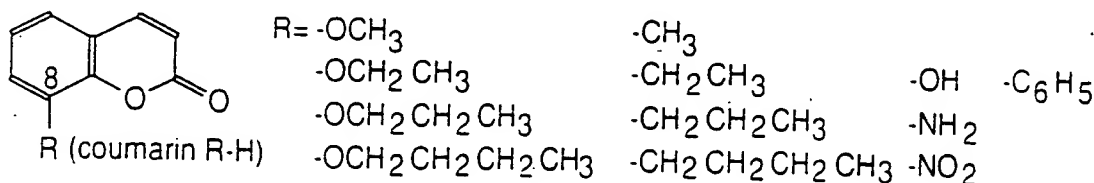
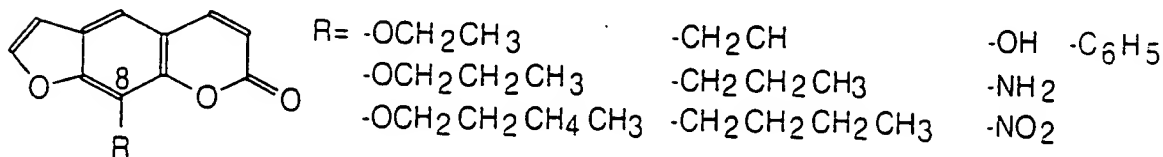
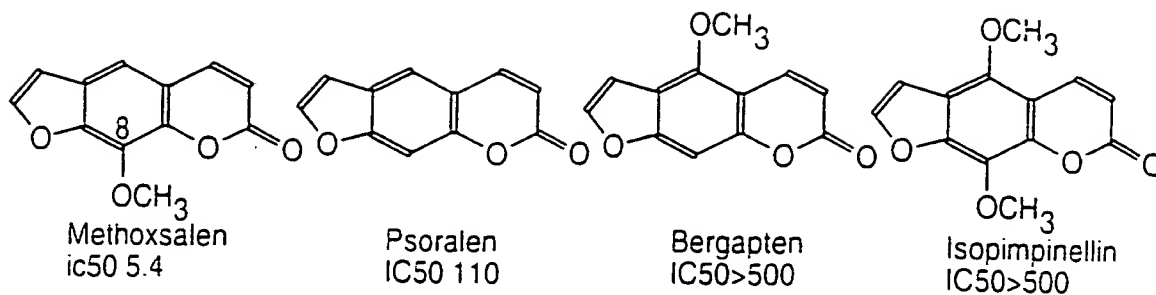


FIG.23A

666060" F5847250

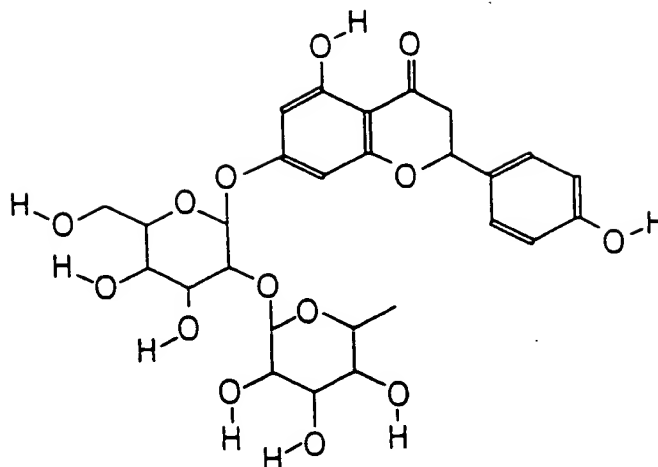
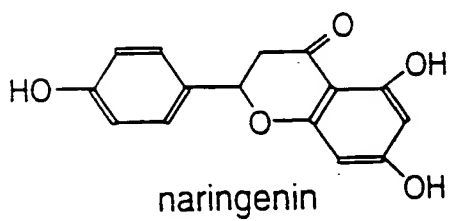




FIG.23B


Oc1cc2ccccc2oc1=O.Cc1cc2ccccc2oc1=O

Dicumarol


  
4-Chromanone

  
4-Chroman


Oc1ccc2ccccc2c1  
1-Naphthol




1,3-Indandione



1-Indanone



Warfarin


  
 Sphondin
   
 IC50 90

Amgelicin  
IC 50 160

**Pimpinellin**  
IC<sub>50</sub>>500

$$(\text{CH}_3\text{CH}_2)_2\text{NCS}_2\text{NH}_4$$

Diethyldithiocarbamic acid ammonium salt

CN1C(=O)SC2=CC=CC=N21
$$[(\text{CH}_3)_2\text{N}]_3\text{P}(\text{O})$$

Hexamethylphosphoramide

$$(\text{CH}_3)_2\text{NNO}$$

N-Nitrosodimethylamine

FIG.23C

## FIG. 24A

The SAS System  
Experiment BCL; Pharmacokinetics of nicotine  
Revised analysis of kinetics based on re-assays  
Does treatment affect AUC?

Compound assayed=COTININE

Dependent Variable: AUC

Source  
Model  
Error  
Corrected Total

Sum of Squares
10578731978
1944298022
12523030000

Mean	Square
151124745	
324049670	

F Value	Pr > F
4.66	0.0397

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AUC Mean  
90876.07

R-Square  
0.844742

C.V.  
19.80871

Root MSE  
18001.38

F Value	Pr > F
5.43	0.0294
0.09	0.7690

Type	I SS	Mean	Square
10548143898	1758023983		
30588081	30588081		

Source			
SUBJ			
DF	6	1	

Least Squares Means

TREATMNT	LSMEAN	AUC
Methoxsalen10-50	92354.2010	
placebo	89397.9447	

FIG. 24B

The SAS System  
Experiment BCL; Pharmacokinetics of nicotine  
Revised analysis of kinetics based on re-assays  
Does treatment affect AUC?

Compound assayed=NICOTINE

Dependent Variable: AUC

Dependent Variable:	AOC	Sum of Squares	Mean Square	F Value	Pr > F
Source	DF				
Model	7	54879492.87	7839927.55	5.14	0.0317
Error	6	9143654.02	1523942.34		
Corrected Total	13	64023146.88			

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AUC Mean  
7165.426

```
Root MSE
1234.481
```

R-Square  
0.857182

C.V.  
17.22829

Pr > F  
0.1422  
0.0038

F Value	2.52	20.86
---------	------	-------

Mean Square  
3847592.43  
31793938.32

Type	I	SS
230855	54.55	
317939	38.32	

DF 6 1

Source  
SUBJ  
TREATMT

Least Squares Means

AUC  
LSMEAN

## TREATMENT

8672.40779  
5658.44323

Methoxsalen10-50  
Placebo

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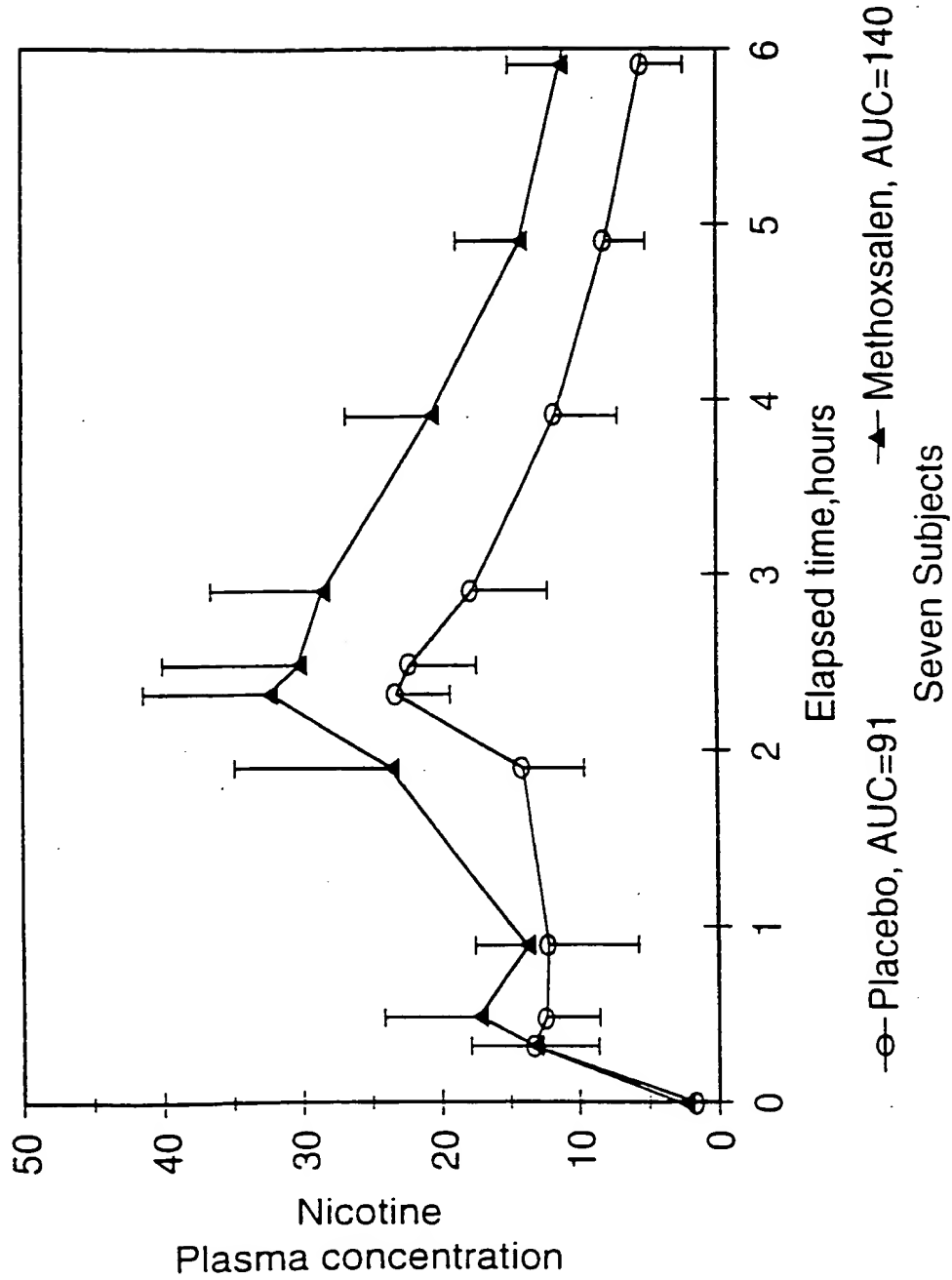


FIG.25



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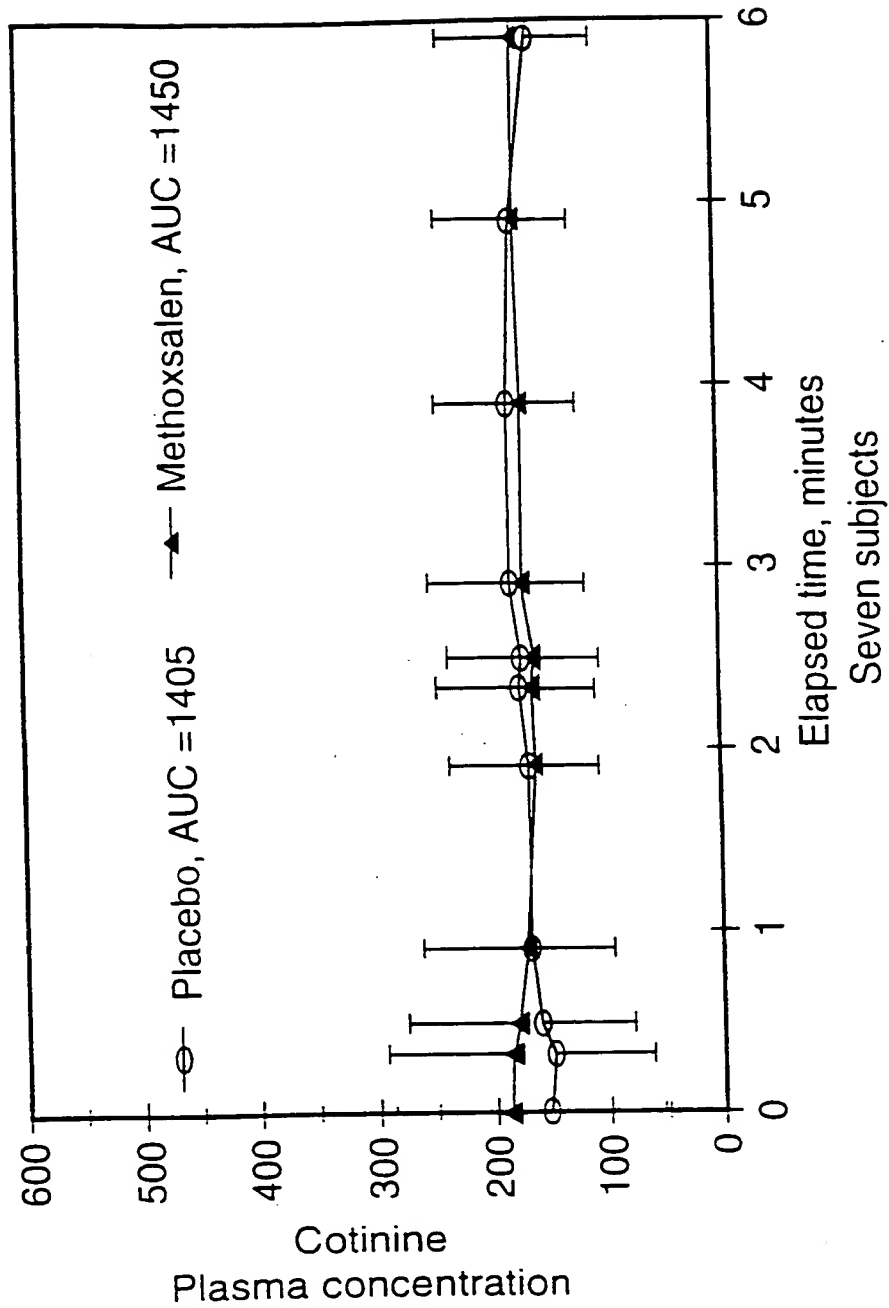


FIG.26

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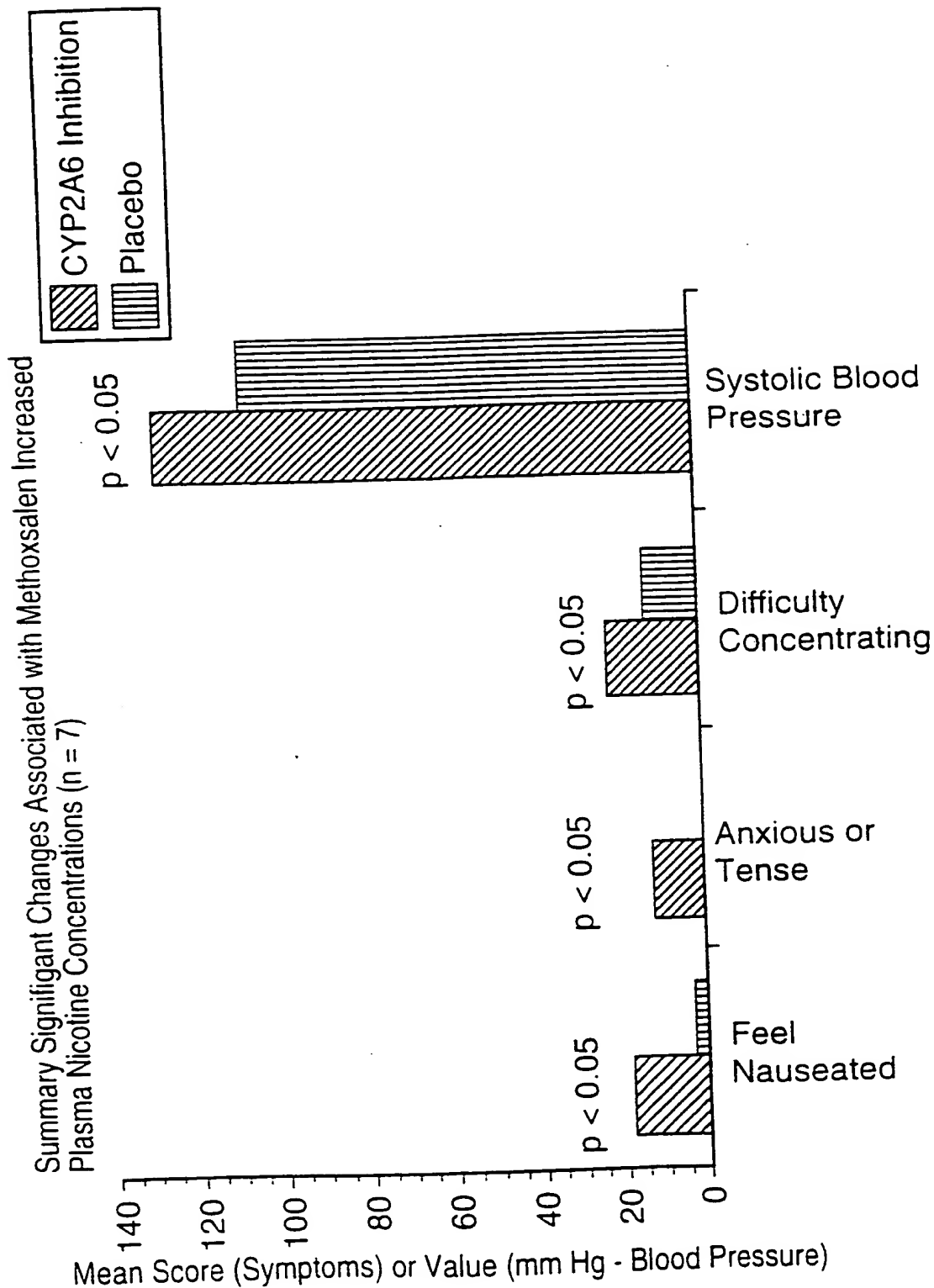


FIG.27

Subjective Rating of Current Nausea (n=7)

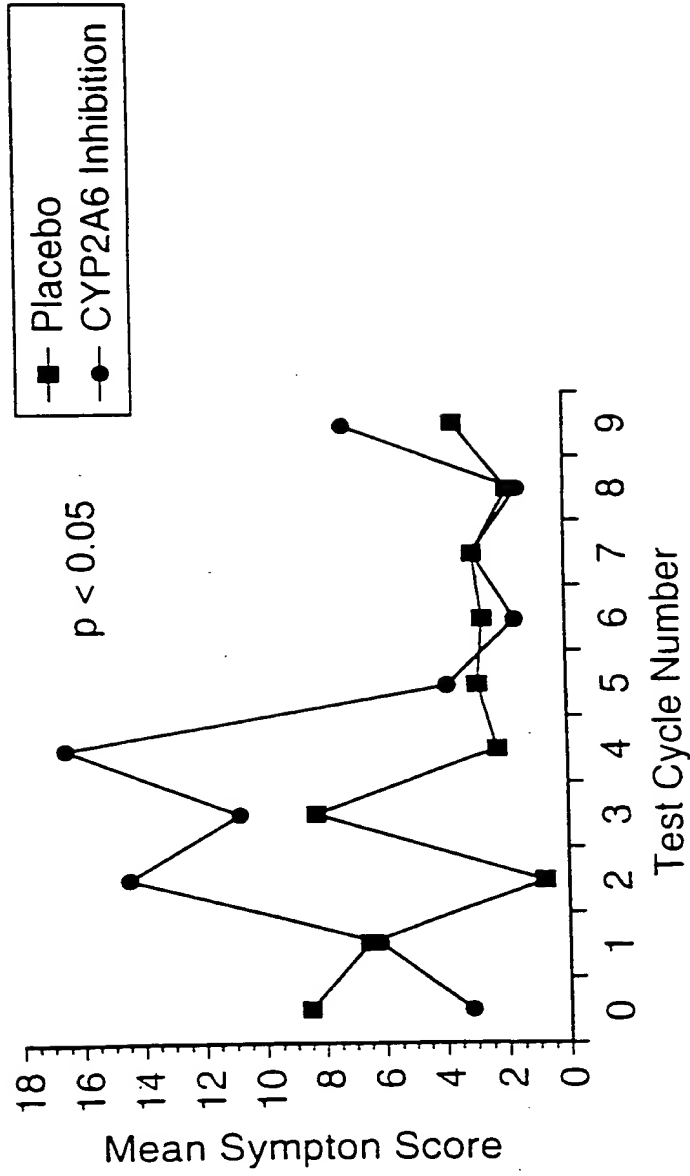


FIG.28A

Subjective Rating Of Current Desire for a Cigarette  
(n = 7)

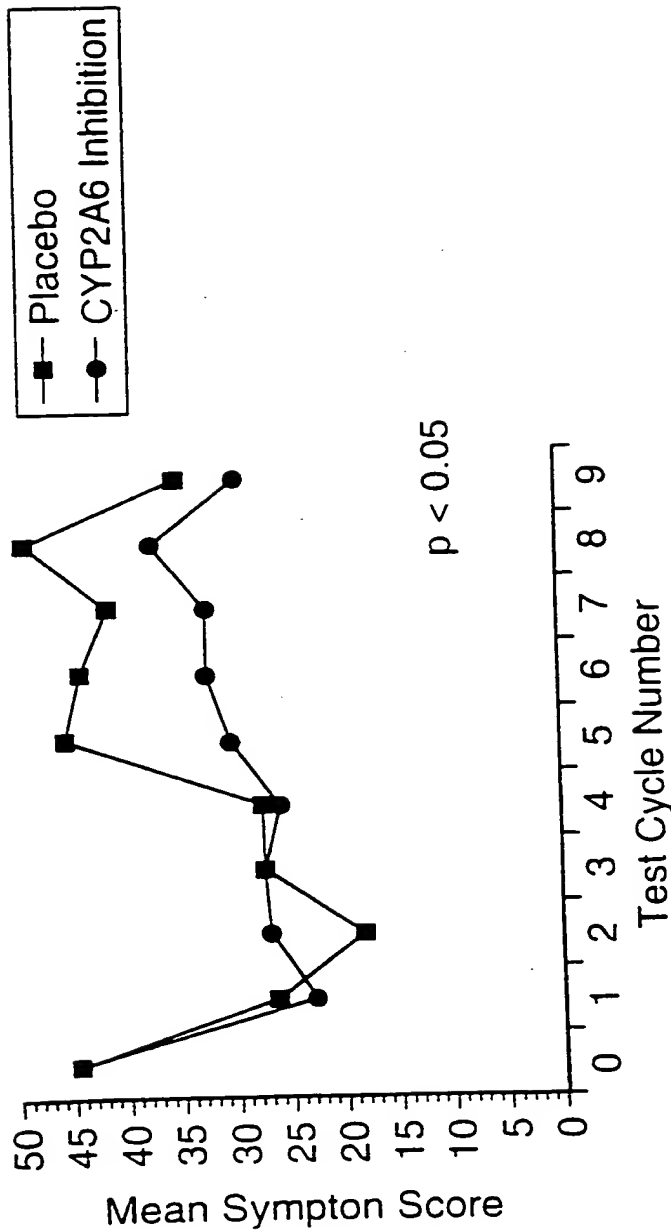


FIG.28B

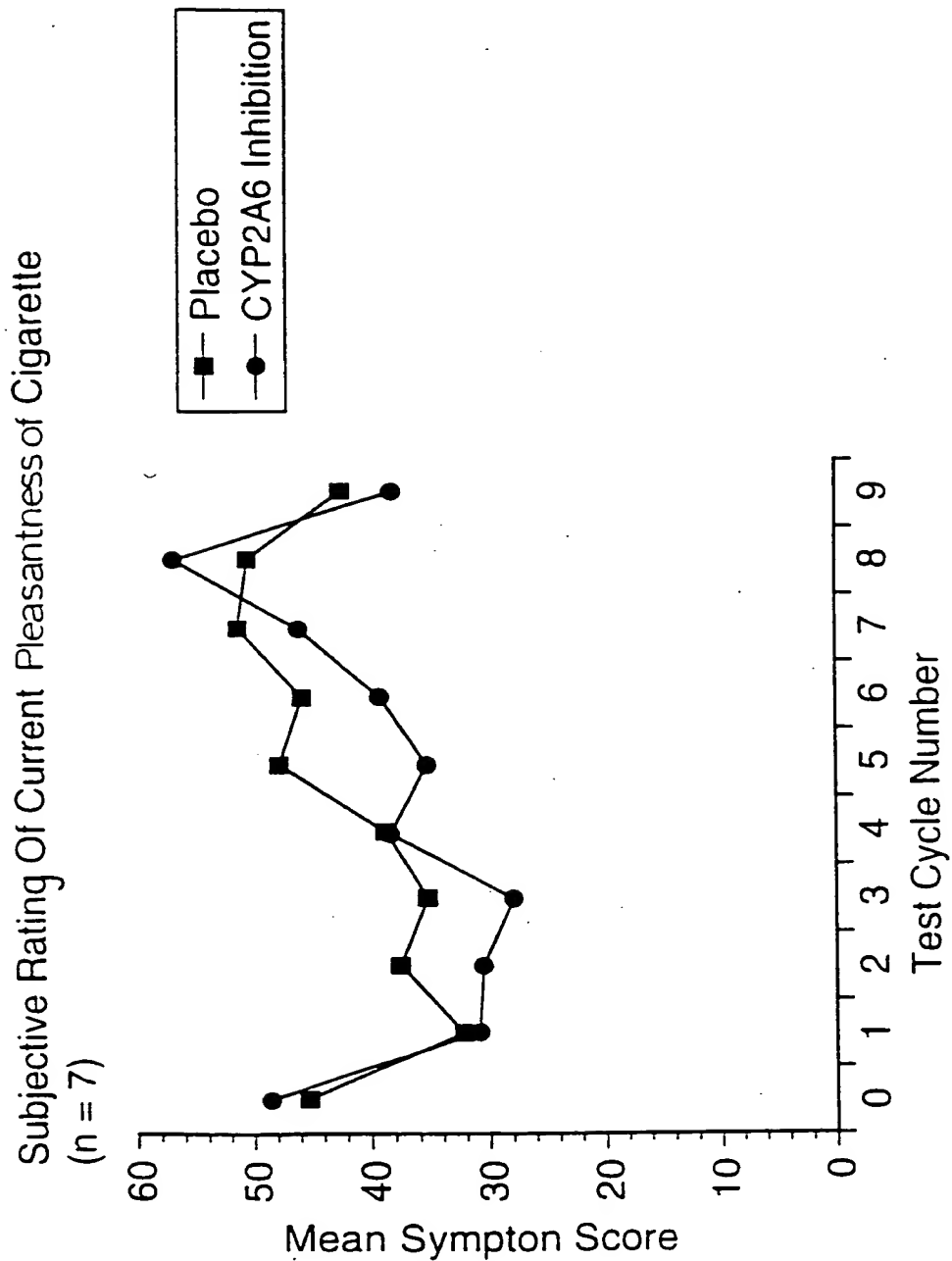


FIG.28C

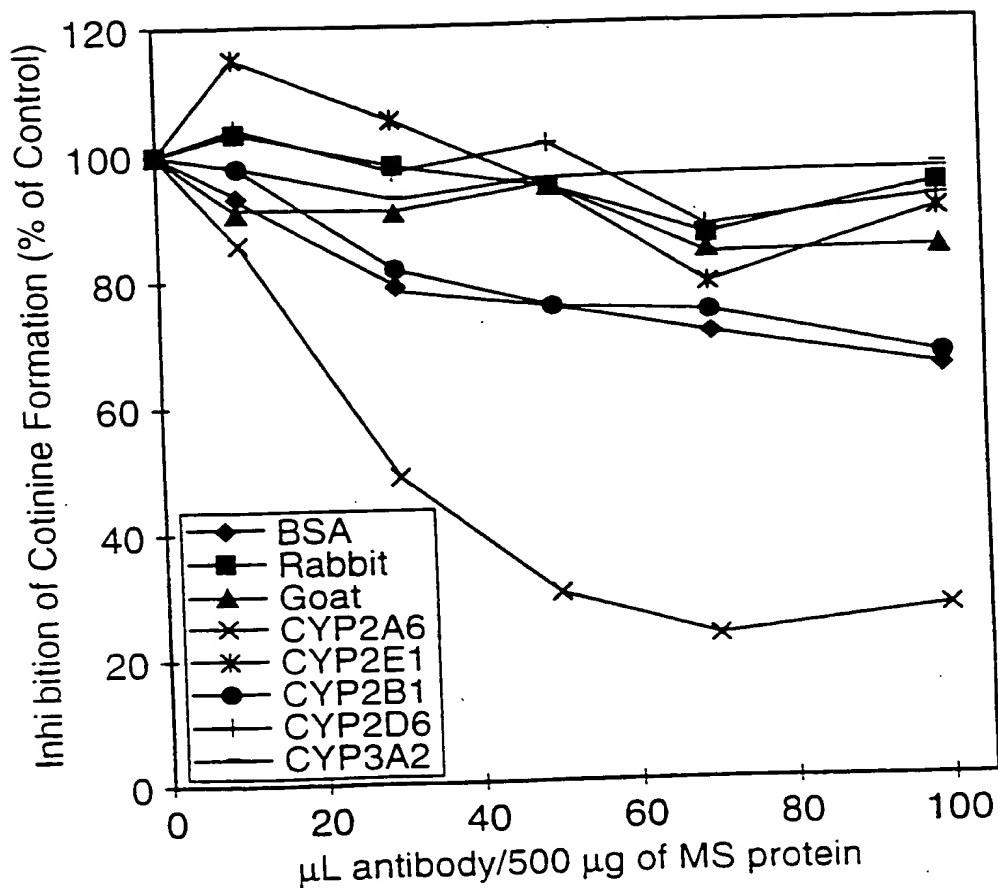


FIG.29

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Inhibition of Nicotine to Cotinine Metabolism by various Compounds

Inhibitor	K <sub>i</sub>	% Inhibition at 10 uM	% Inhibition at 100 uM	% Inhibition at 150 uM
coumarin	2 uM (n=4)	65 (n=1)	90 (n=1)	85 +/- 11 (SD, n=31)
7-methoxycoumarin	2.5 uM (n=1)	40 (n=1)	60 (n=3)	—
7-methylcoumarin	15 uM*	20 (n=1)	70 (n=3)	—
7-ethoxycoumarin	>100 uM*	10 (n=1)	20 (n=3)	—
7-hydroxycoumarin	200 uM	—	25 (n=1)	—
diethylthiocarbamic acid	14.5 uM (n=1)	—	—	—
pilocarpine	0.1 uM	—	—	—
naringenin	4.3 uM (n=1)	30 (n=1)	70 (n=3)	—
methoxsalen	0.02 uM (n=1)	—	—	—
naringin	100 uM*	—	10 (n=1)	—
bupropion	—	20 (n=1)	30 (n=1)	—
orphenadrine	—	—	—	20 +/- 16 (SD, n=30)
troleandomycin	—	—	—	3 +/- 11 (SD, n=30)

all nicotine concentrations were at the K<sub>m</sub> value for cotinine formation in their respective livers  
\* estimated from screening studies with 10 and 100 uM inhibitor concentrations

FIG.30A

K<sub>i</sub> Values for the Inhibition of the CYP2A6 Substrate Coumarin to 7-Hydroxycoumarin Metabolism by various compound

Inhibitor	Human liver	Monkey liver
methoxsalen	0.29 uM	1.69 uM
nicotine	100.1 uM	24.1 uM
pilocarpine	0.9 uM	0.9 uM

FIG.30B



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Effect of Various Compounds on Cotinine Formation  
% control cotinine formation

Inhibitor	10 $\mu$ M	100 $\mu$ M
coumarin	35	10
naringenin	70	30
7-methylcoumarin	80	30
7-methoxycoumarin	60	40
bupropion	80	70
7-ethoxycoumarin	90	80

FIG.30C

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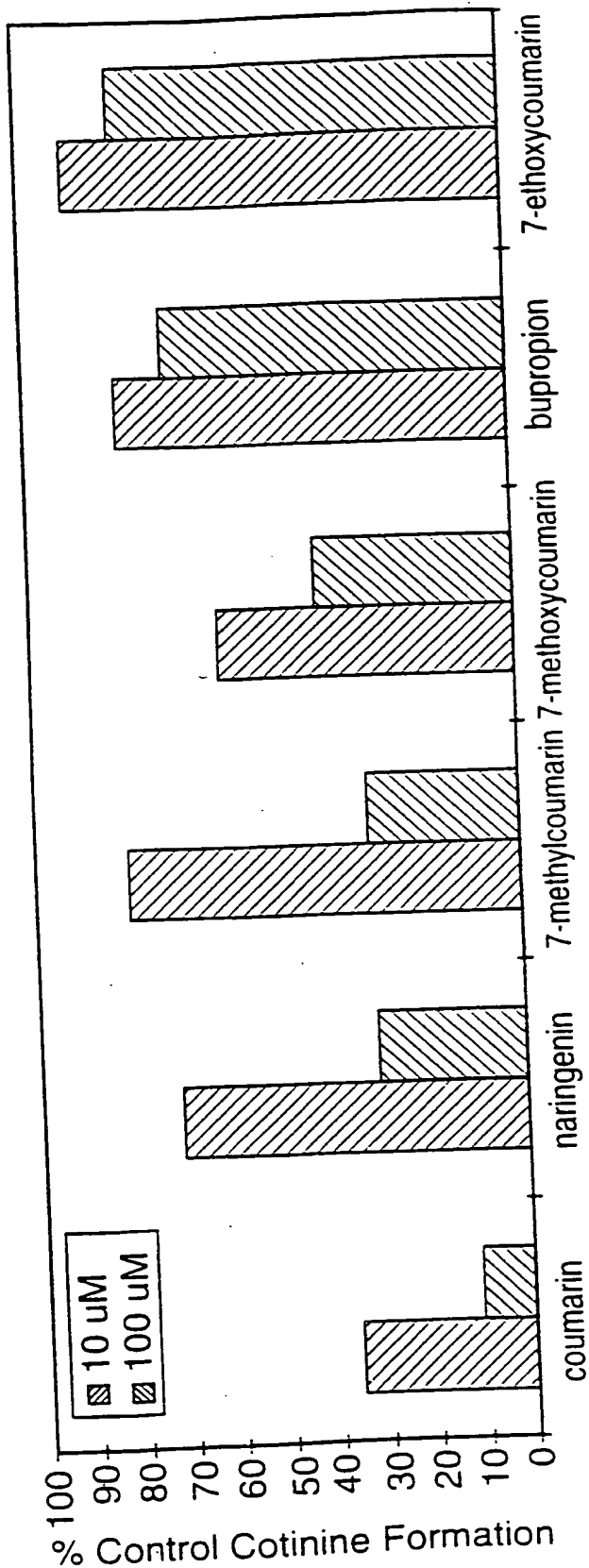
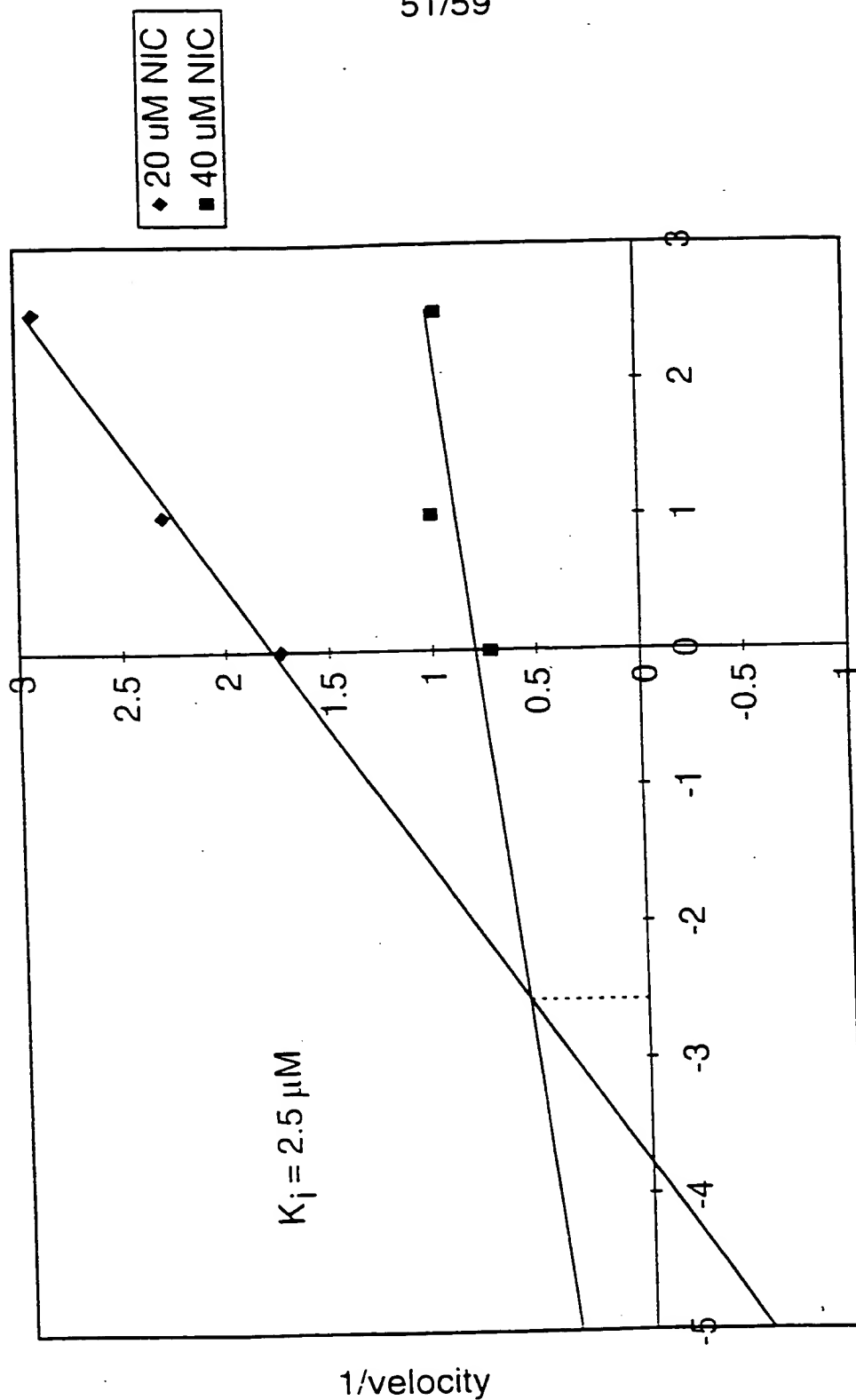


FIG.30D

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Dixon Plot of 7-Methoxycoumarin Inhibition of Nicotine to Cotinine Formation in K28 Human Liver Microsomes



7-methoxycoumarin Concentration ( $\mu\text{M}$ )

FIG.31

Dixon Plot of Methoxsalen Inhibition of Nicotine to Cotinine Formation with 10 Minute Preincubation in K28 Human Liver Microsomes

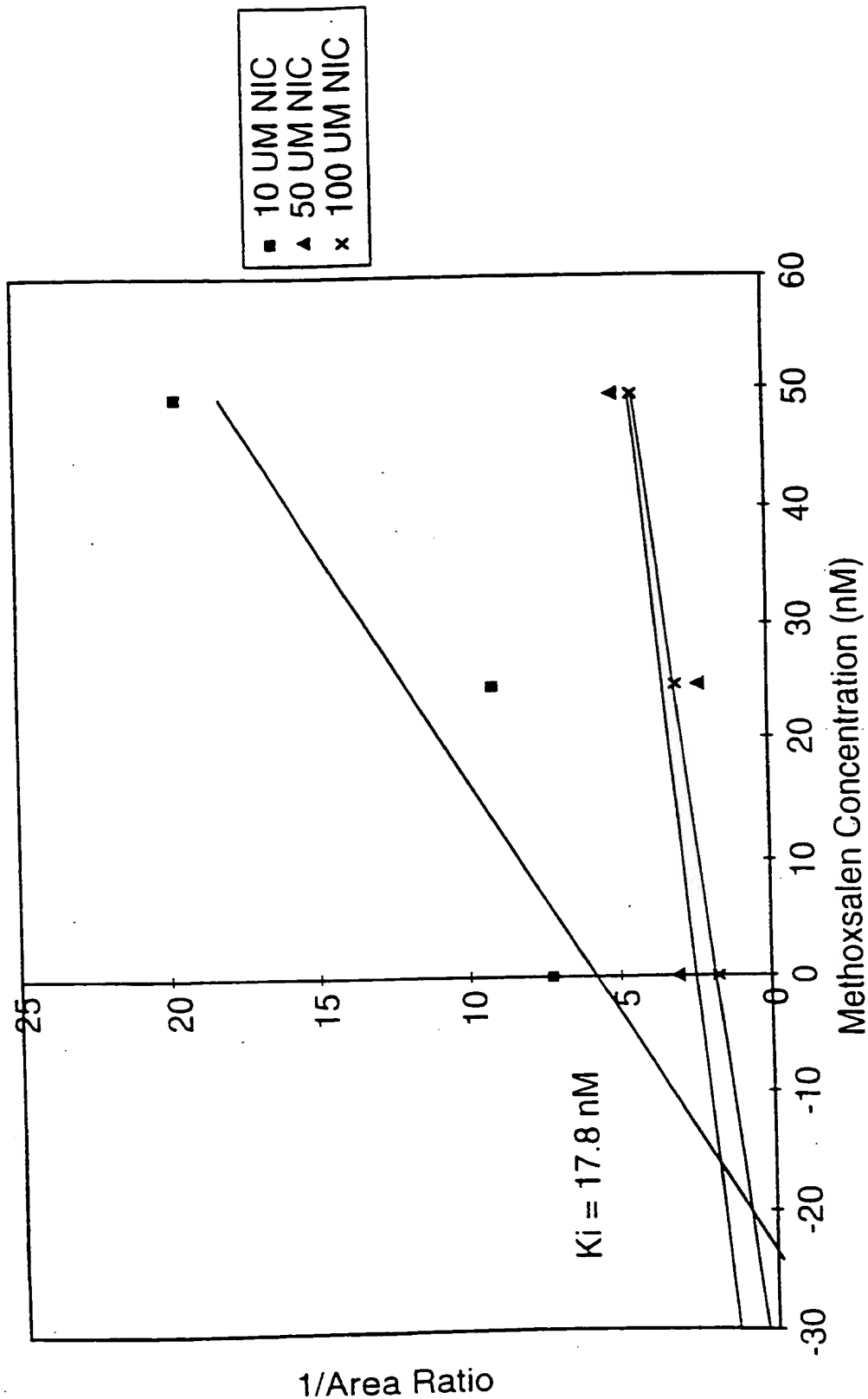


FIG.32

Cornish-Bowden Plot of Methoxsalen Inhibition of Nicotine to Cotinine Formation  
with 10 Minute Pre-incubation in K28 Human Liver Microsomes

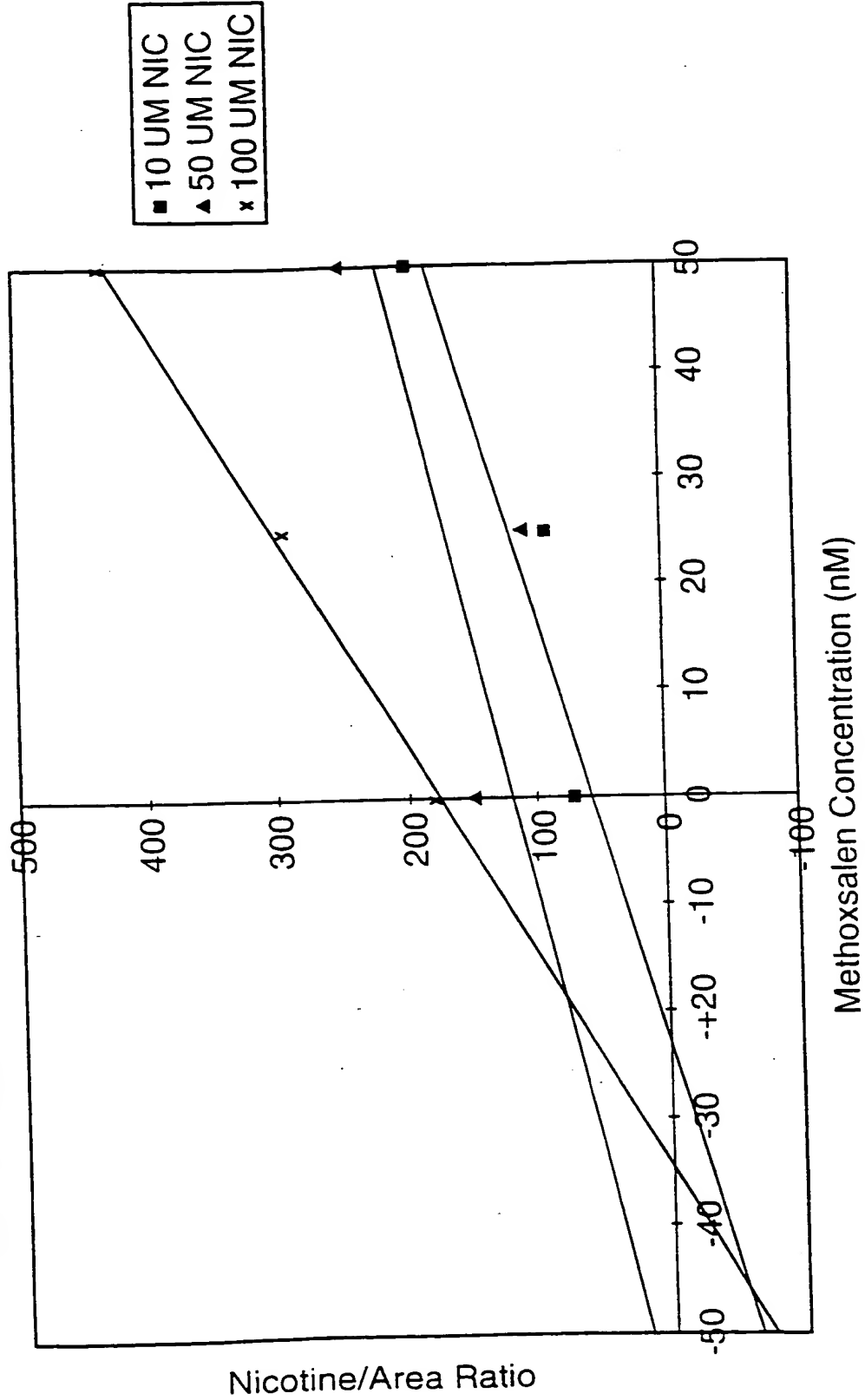


FIG.33

Effect of Pre-incubation Time of Methoxsalen (100 nM) on the Inhibition of Nicotine (30  $\mu$ M) to Cotinine Formation in K26 Human Liver Microsomes

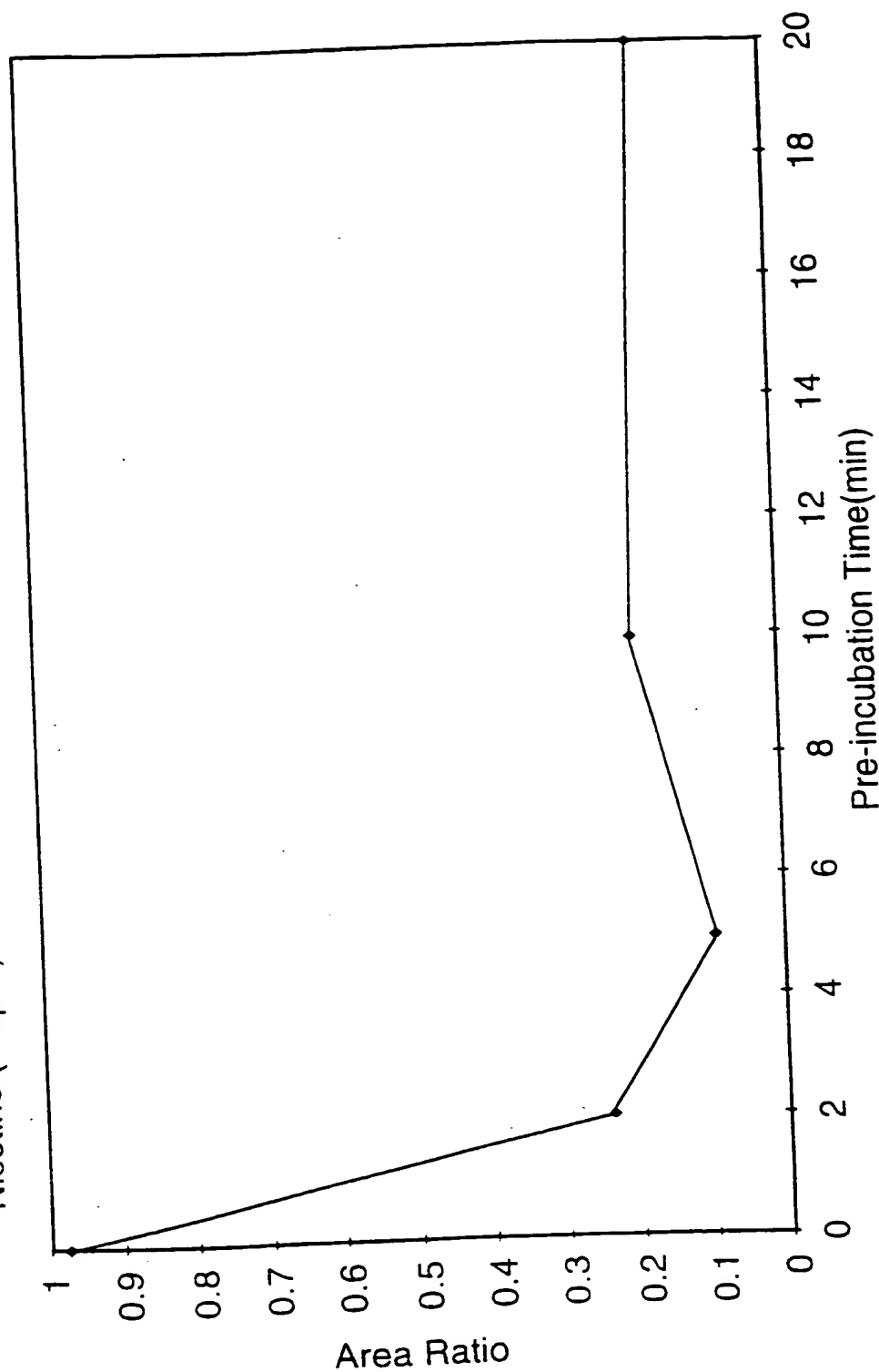


FIG.34

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Dixon Plot of Naringenin Inhibition of Nicotine to Cotinine Formation with 10 minute  
Preincubation in K26 Human Liver Microsomes

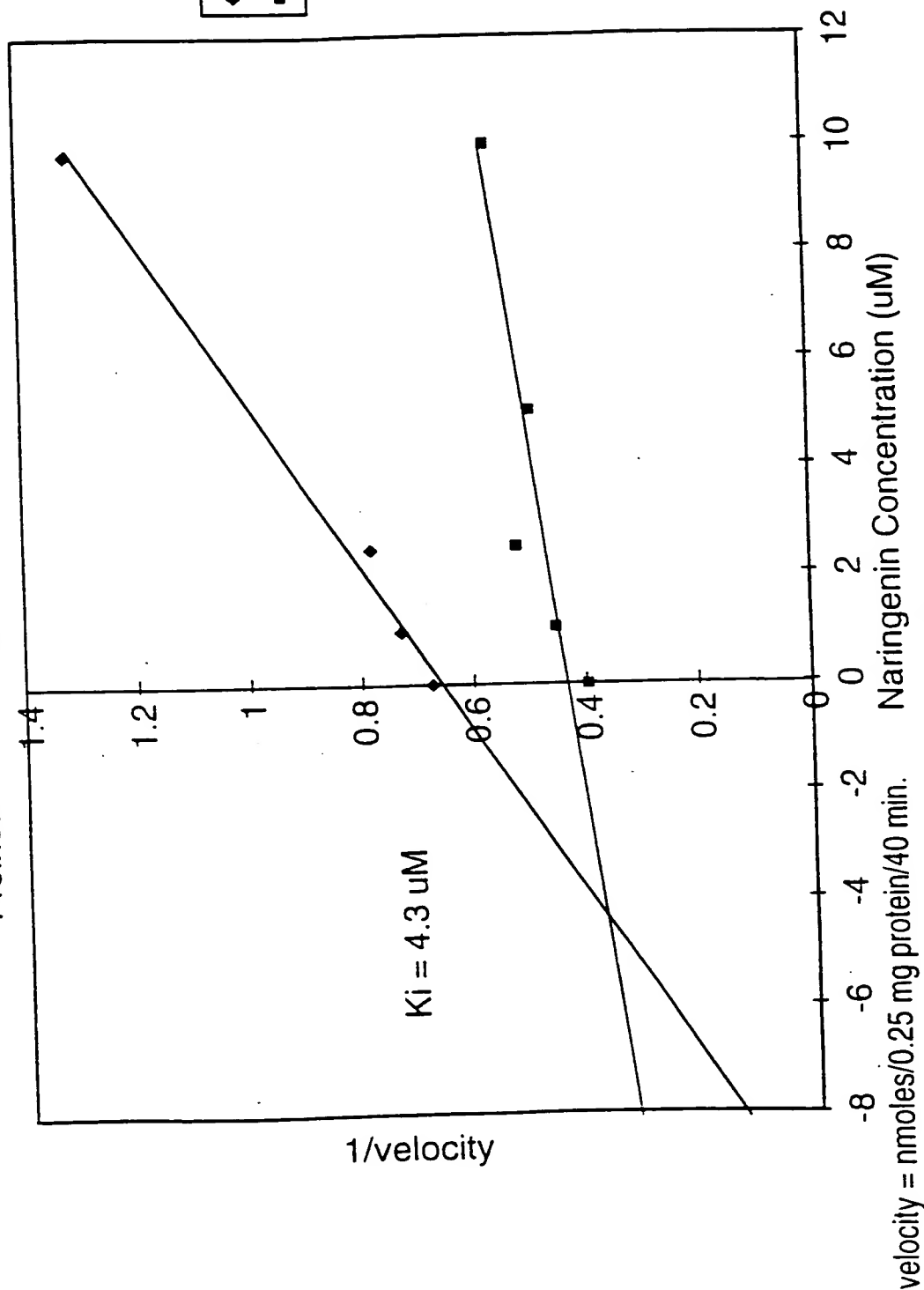


FIG.35

Dixon Plot of Diethyldithiocarbamic Acid Inhibition of Nicotine to Cotinine Formation with 10 Minute Preincubation in K26 Human Liver Microsomes

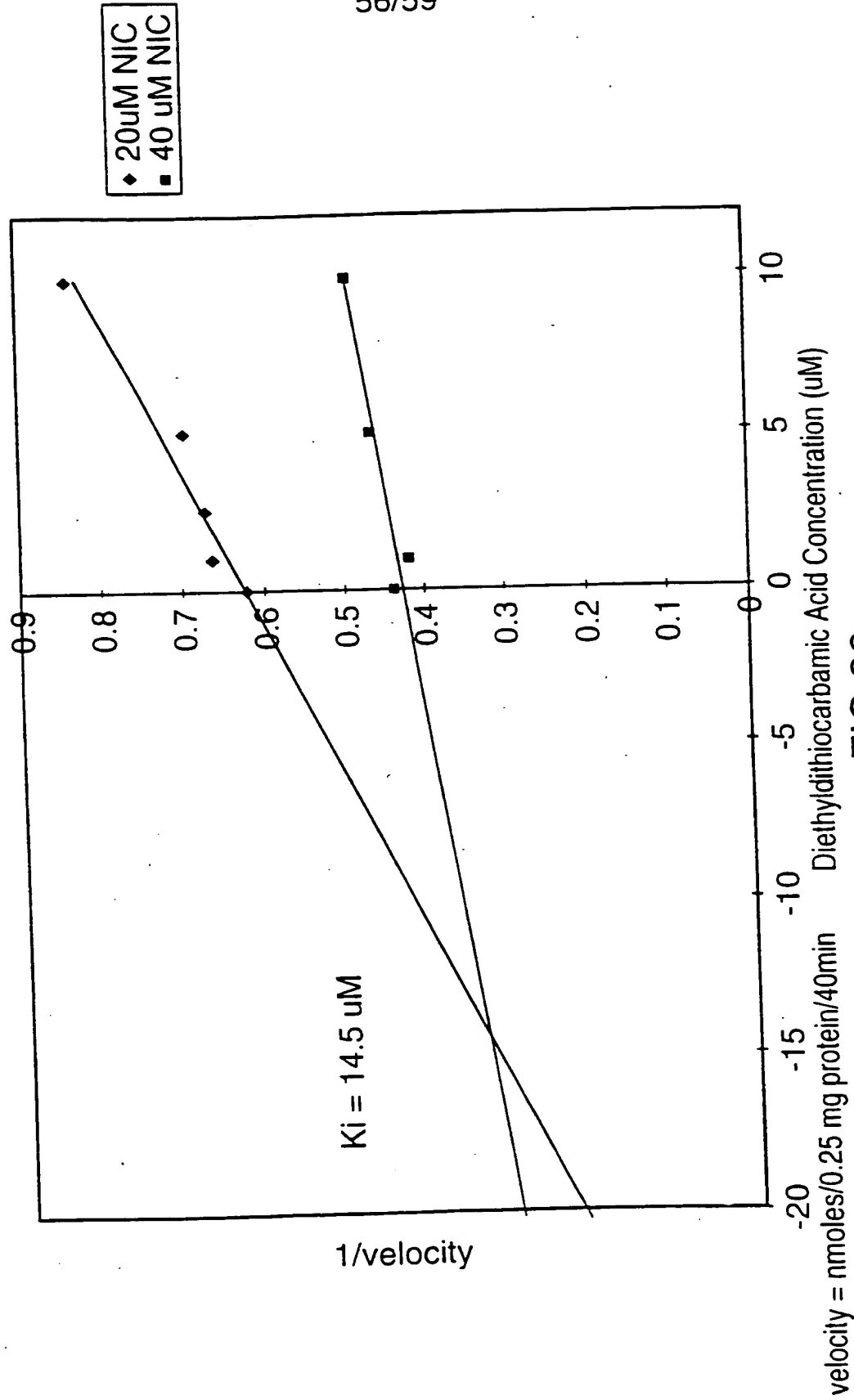


FIG.36



# Comparision Between Morning and Afternoon Testing Sessions

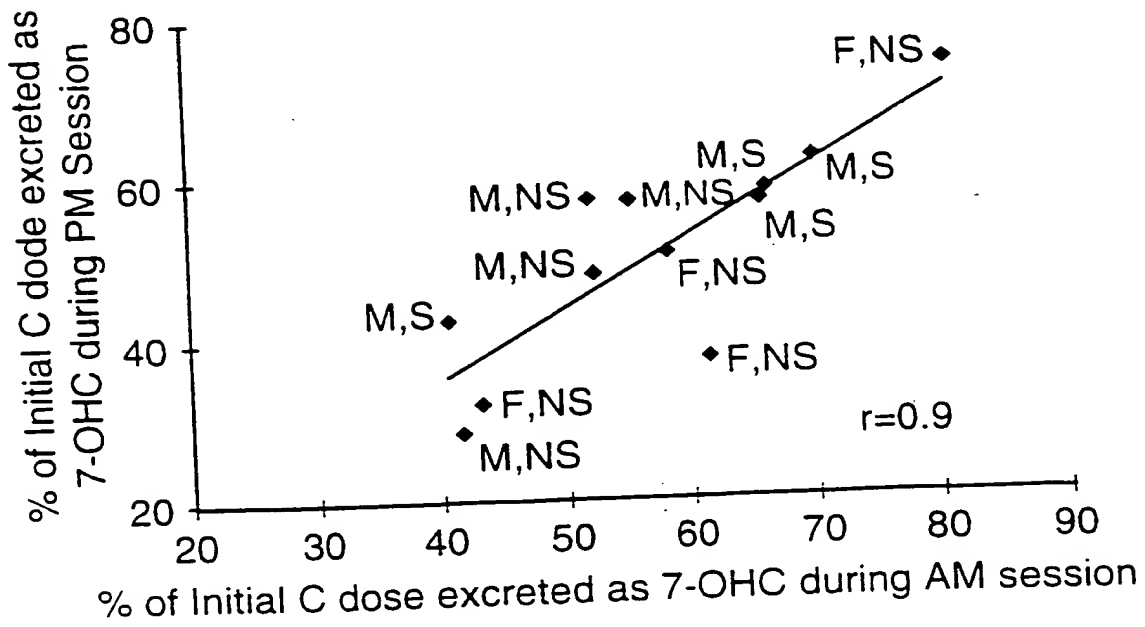


FIG.37

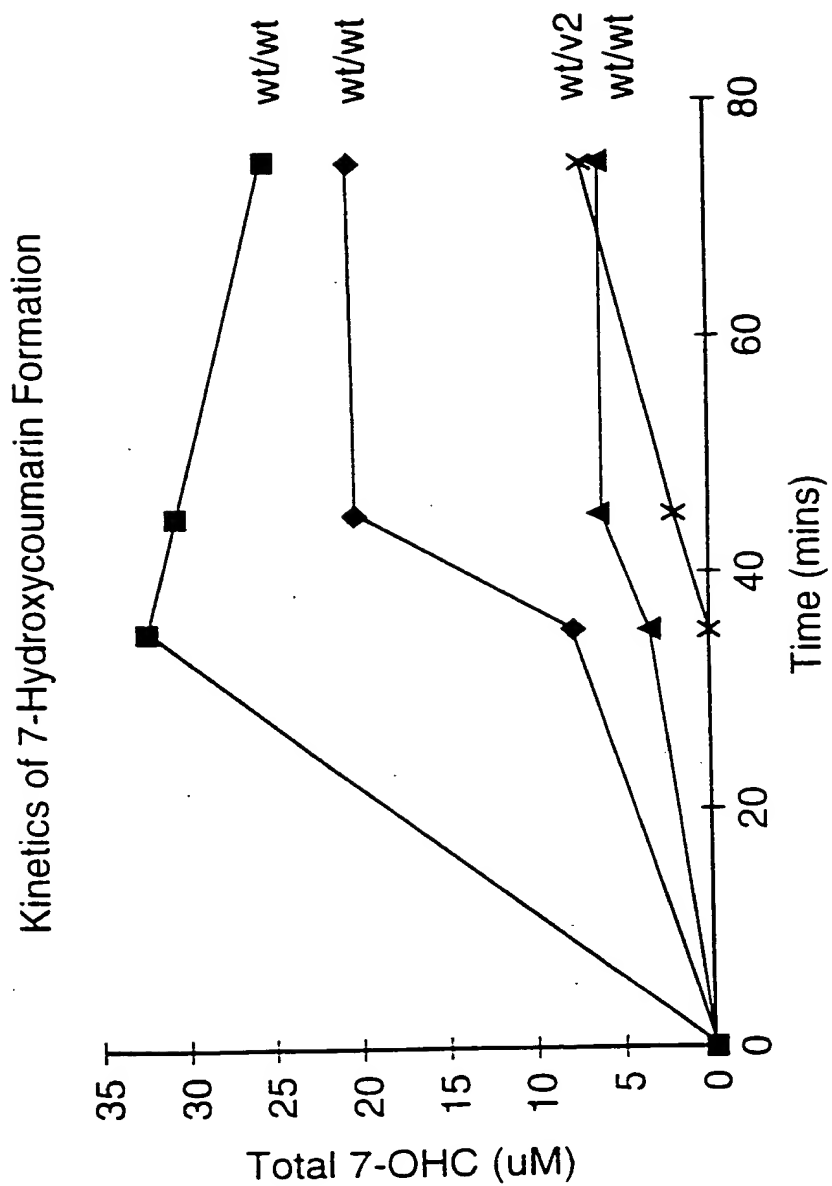


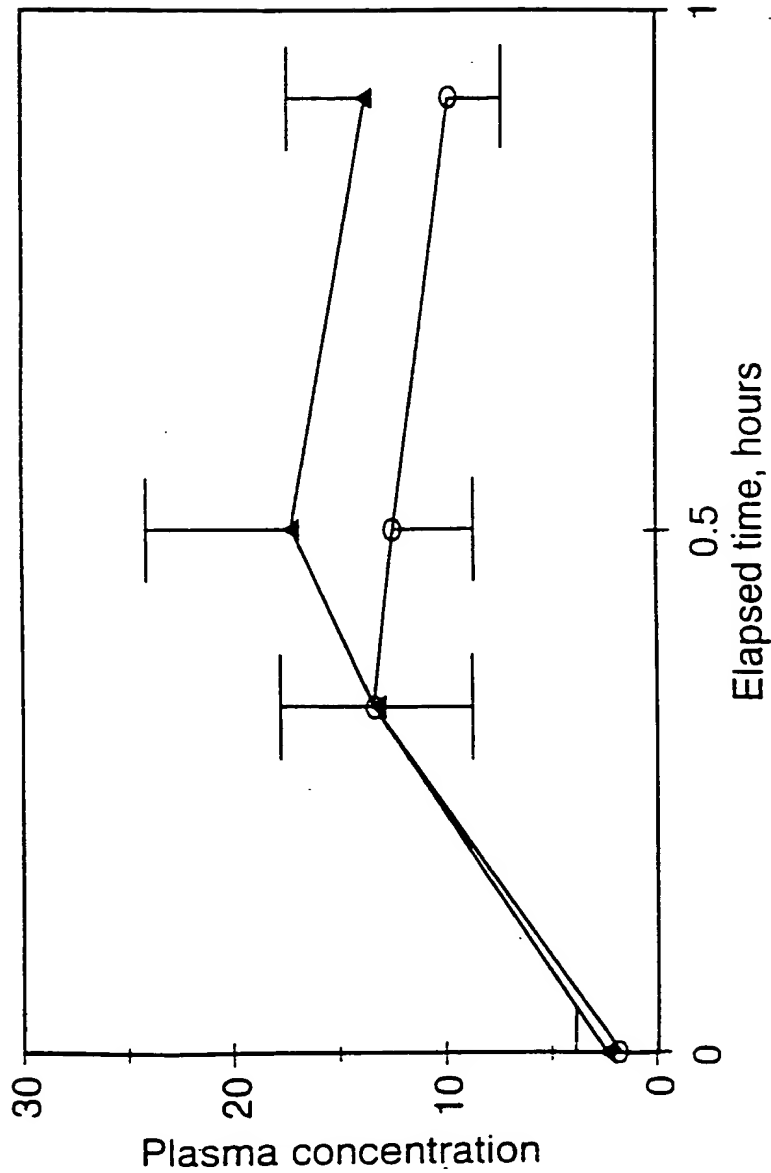
FIG.39

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566060" TEST 250

Metabolism of Nicotine over one hour  
Mean and s.d., seven subjects



○ Placebo, AUC=12      ▲ Methoxsalen, AUC=15

Placebo vs Methoxsalen AUC to 1 hour:

$F(1,6)=8.07, p=0.0295$

FIG.38